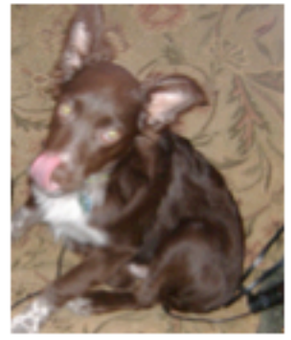
Identification of Bacteria from Dog Paws and a Book Bag

Jaime Campbell, Olga Harris, Robert Burden, David Mitchell



Abstract

Bacteria are ubiquitous and are found on nearly every surface humans come in contact with daily. In an effort to look at non-deliberate ways for individuals to come in contact with potentially harmful bacteria, this experiment attempted to isolate and identify four distinct bacterial cultures from both a student's bookbag and the bottom of a dog's paw. Various tests were performed on each isolate to give characteristic information in an effort to identify the genus and species. These tests included numerous morphological stains, differential and selective medias, oxygen requirements, 16S rRNA sequencing, temperature and pH dependence and a number of biochemical and physiological tests. These tests helped to identify the genus of each of the isolates. The samples Dog 5 was found to be gram positive aerobic rods that was catalase positive and utilized glucose fermentation leading us to believe the isolate was of the genus *Bacillus.* Bookbag 4 was an orange culture that only grew at 25°C. The isolate was polymorphic and non-spore producing. Bookbag 4 was determined to be *Paracoccus.* In conclusion, four bacteria were successfully isolated and characterized to the genus level from the two environments of interest.

Introduction

Bacteria are ubiquitous and are found on nearly every surface humans come in daily contact with. The vast majority of bacteria are harmless to humans; however, there is a percentage that is pathogenic and potentially dangerous to human health. It is therefore important for individuals to understand bacteria and be aware of possible bacteria they may encounter everyday. In an effort to look at non-deliberate ways for individuals to come in contact with potentially harmful bacteria, this experiment examined two environments; a student's book bag and the bottom of a dog's paws.

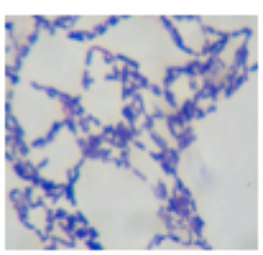
The primary source for bacteria isolated from a dog's paw is soil, due to their exposure to the ground when taken on walks. Several types of bacteria that are common in the soil is *Pseudomonas, Arthrobacter, Clostridium, Bacillus, Micrococcus,* and *Chromobacterium* (Rangaswami, 2005).

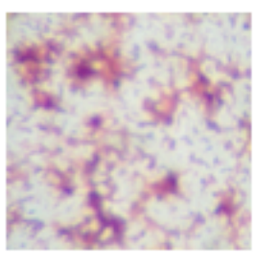
Another environment this study focused on was the bottom of a bookbag. Countless students use bookbags every day. They are thrown under desks, dropped on dinning hall floors, rested on kitchen counter tops in homes, and even set on floors in public restrooms. Common species found in these environments that could be isolated include *Escherichia coli, Enterobacter, Klebsiella, Proteus, Pseudomonas, Salmonella, and Shigella* (Madigan, 2006).

In this study we attempted to isolate and characterize two species of bacteria found in the presented environments in which humans come in contact with on a daily basis. We attempted to characterize the isolates through a number of tests in hopes of identification to the genus and species.

Methods

- Pure colonies were isolated from a book bag and dog's paws using sterile swabs and PBS.
- **Differential test**: MR-VP, Catalase, Oxidase, Nitrate reduction, Blood agar, SIM media, TSI agar, and MSA plates
- **Selective test**: Gram stain, Endospore stain, MSA plates, and EMB plates
- **Growth test**:pH, temperature, agar deep stabs, and fluid thiogycollate media
- **Special test**: Citrate test, PCR amplification of 16S rRNA gene





Gram stains of isolates Dog 5 (left) and Book bag 4 (right).

Results

	Dog 5	Book Bag 4
Description	Large CFUs, round, irregular, yellow, convex	Very small CFUs, round, smooth, orange
Gram +/-	+	-
Shape	Rod (chains)	Polymorphic
Size	Small	Small
Spores	None present	N/A
PCR success	Clean band at 1500 bp	Band at 1500 bp
Fluid Thioglycolate	Facultative aerobe	Strict aerobe
Agar Deep	Facultative aerobe	Strict aerobe
Mannitol Soy Agar	Inhibited by NaCl	N/A
EMB Plates	N/A	- Eosin/ - methylene blue
Blood Agar Plates	β hemolysis	γ hemolysis
Nitrate Reduction	Nitrate Reduction	No Nitrate Reduction
SIM Media		
-Sulfur Reduction	-	-
-Tryptophan breakdown	-	-
-Motility	motile	non-motile
TSI*	K/A	NC/NC
Catalase Presence	+	+
Oxidase Presence	+	-
MRVP		
-Methyl Red	+	-
-V.p	-	-
Optimal pH	7	7
Optimal Temperature	37°C	25°C
Citrate	N/A	-

The Dog 5 isolate was found to be a gram positive rod arranged in chains. It was a non-spore producing aerobe that was catalase and oxidase positive. Its optimal growth occurred at 37°C, pH 7 and could perform β hemolysis. The Bookbag isolate was an orange, gram negative polymorphic culture. It was non-motile and grew optimally at 25 °C and pH 7. The isolate was oxidase and citrate negative and catalase positive.

* Symbols on chart
A/A: glucose and lactose and/or sucrose fermentation with acid accumulation
K/A: glucose fermentation with acid production
NC/NC: No fermentation/ not Enterobacteria
N/A: Not applicable to that isolate

Discussion

The isolate D5 was found to be from the genus *Bacillus.* Bacteria found in the genus *Bacillus* are primarily found in soil and have high G+C content (32-69%) (Holt, 2000) This would correlate with our findings due to Dog's paw constantly being in contact with the soil. The bacteria of this genus are normally spore producing. This isolate did not produce spores, which could be due to experimental error or that some species take up to 7 days for spores to form. The spore stain may have been performed on samples too young for spore production to have occurred. Spores form in organisms when nutrients are not readily available and as the cultures get older. If there were sufficient nutrients available to the organism, there would be no need to form spores therefore explaining our negative result. *Bacillus* bacteria are primarily catalase negative, which is supported by our findings in this isolate. Some species can contain flagella, which would contribute to the motility observed in the D5 isolate (Holt, 2000). Overall, our finding support the prediction that D5 is a member of the genus *Bacillus.*

BB4 was found to be a member of the genus *Paracoccus*. This is supported by the characteristics of our isolate. Like *Paracoccus*, BB4 is a gram negative, non-motile, aerobic bacteria that is catalase positive. The optimal temperature for growth was 25°C which agrees with the 25-30°C optimal growth for the genus *Paracoccus* (Holt, 2000). Also, the shape was found to be polymorphic, including what appears to be both rods and cocci like cells. This coincides with *Paracoccus* genus because its typical cell morphology is spherical cells or short rods. One would expect polymorphic cells to be present from the genus. One disagreement would be that normally species found in this genus are oxidase positive and our isolate was oxidase negative. This could be due to experimental error, or just by variability in the different species isolated.

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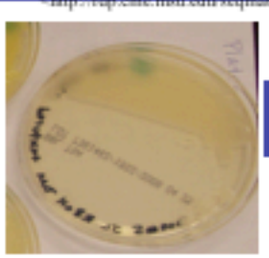

Original cultures of Dog 5 (left) and Book bag 4 (right).

Identification of Isolates from Ferret Feces & Ground Beef

Atara Taylor, Trey Woolwine, Whitney Davis, Kat Kross

Summary

Unknown bacteria from two environments, ferret feces and raw ground beef, were cultured and characterized. The most likely identities of the organisms are *Enterococcus faecium* and *Citrobacter freundii*, respectively.

Introduction

There is an abundant natural microbial flora that exists in common environmental locations and in animal hosts. The purpose of this investigation was to isolate, characterize, and ultimately identify bacterial species from a variety of environments. The specific environments chosen for study were raw ground beef and ferret feces.

Escherichia coli has been directly isolated from raw ground beef (Saad, 1999). *E. coli* is a gram negative rod, and there are a number of strains, but only one, O157:H7, is a known pathogen. *Bacillus cares*, *Bacillus circulans*, and *Bacillus licheniformis*, all of which are gram-positive rods are also associated with raw meats (Pawsey, 2002).

Another environment of study, ferret feces, consists of a sample of 'fresh' feces obtained from a domestic ferret. Ferrets are strict carnivores, like cats, and so the following bacterial flora might be expected in a ferret fecal sample: *Bacteroides*, *Eubacterium*, *Pentostreptococcus*, *Enterococci*, coliform bacteria, *Lactobacillus*, and Welch's bacilli (Mitsuoka, 1978). This flora largely overlaps with other animals, but lacks the *Bifidobacterium* that is common to other monogastric animals (Frank, 1992), most likely because of the physiological differences in a strictly omnivorous animal's digestive tract.

Methods

Samples of ferret feces and raw ground beef were suspended in phosphate buffered saline (PBS) and cultured on Tryptic Soy Agar (TSA) plates to obtain isolated bacterial colonies. The following diagnostic tests were performed on the bacterial isolates, as described by LaRoffe and Pierce (2002), Bourgalt (1988), and Temple (2008):

- Gram stain
- KOH
- Wet mount
- SIM medium
- TSI
- Agar deep stab tubes
- Thioglycollate tubes
- Growth at optimum pH and temperature
- MSA plate
- EMB plate
- MR-VP test
- Catalase
- Oxidase
- Nitrate reduction
- Blood agar
- Coagulase
- PCR isolation and sequencing of 16s rRNA gene

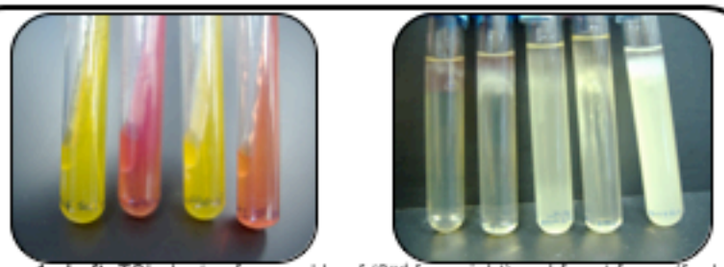

Figure 1. Left: TSI slants of ground beef (2nd from right) and ferret feces (far left) isolates. **Right:** Thioglycollate tubes of ground beef (middle) and ferret feces (2nd from right) isolates.

Results

Table 1. Results of various tests on bacterial isolates.

Test	Ground Beef	Ferret
Gram Stain	Gram-Negative Bacilli	Gram-Positive Cocci
KOH	+	-
Wet mount test	Motile	Not motile
SIM media motility	Radiating growth	No radiating growth
Agar deep stabs	Growth at top	Uniform growth
Fluid thioglycollate medium	Growth throughout entire tube	Growth throughout entire tube
Optimum pH	9	9
Optimum temperature (°C)	37	37
MSA plates	N/A	No growth, inhibited by NaCl
EMB plates	Abundant growth; purple with metallic green sheen	N/A
Methyl red	Red	Red
Voges-Proskauer	Yellow	Red
Catalase	No bubbles	No bubbles
Oxidase	No color change	No color change
Nitrate reduction	Turned red after reagents added, no addition of zinc	Turned red after addition of zinc
SIM sulfur reduction	No black color	No black color
SIM indole test	Negative	Negative
TSI	Turned yellow throughout entire tube	Turned yellow throughout entire tube
Blood Agar	Grew with small clearings around colonies and greenish coloration	Simple growth with no change in medium
Coagulase test	N/A	No clumping observed

The ground beef isolate is a motile gram-negative bacillus. It is likely a facultative anaerobe, can grow in the presence of eosin and methylene blue, and ferments glucose, lactose and/or sucrose with acid production. The isolate can reduce nitrate, is α-hemolytic, and is a mixed acid fermenter. It is a alkalophile and prefers a 37°C incubation temperature.

The ferret feces isolate is a non-motile gram-positive cocci. It is a facultative anaerobe, inhibited by NaCl, and a mixed acid fermenter. This isolate ferments glucose and converts acid products to acetoin then 2,3-butanediol. Also, it ferments glucose, lactose and/or sucrose, accumulating acid.

Conclusions

Based on the BLAST results of the 16s rRNA gene from the isolate obtained from raw ground beef, the organism is most likely *Citrobacter freundii*. The BLAST indicated an 89% match. *Citrobacter freundii* is a gram negative bacillus that is often found in soil, water, sewage, and food. Additionally, organisms from this genus are motile, facultatively anaerobic, ferments mixed acid, does not ferment glucose to 2,3-butanediol, and does not contain oxidase; all of which is in accordance with the results of differential tests that we performed on this isolate (Holt, 1994). Although *Citrobacter freundii* is part of normal intestinal microflora, this organism has been associated with some outbreaks of diarrhea (Schmidt, 1993).

Our second unknown isolate, obtained from ferret feces, was likely *Enterococcus faecium*. Remarkably, the BLAST database indicated a 100% match with the 16s rRNA gene sequences of this species. Members of the *Enterococcus* genus commonly inhabit vertebrate intestines but are also the third most common cause of infections acquired during hospitalization (Ledeboer, 2007). Our isolate shared characteristics identified in Bergey's Determinative Manual of Bacteriology for this genus: gram positive coccus, facultatively anaerobic, no endospore formation, catalase negative, and optimum growth at 37°C and pH 9.6 (Holt, 1994).

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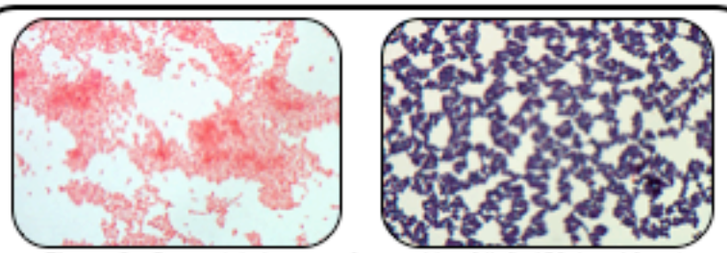
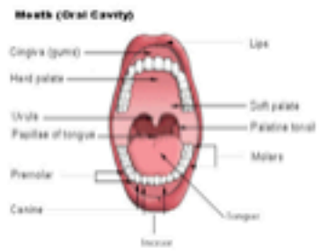


Figure 2. Gram stain images of ground beef (left, 150x) and ferret poop (right, 150x) isolates.



Microbial Diversity in the Human Oral Cavity and a Water Source

Sara Caldwell, Charles Hall, Rob Lera, and Brendan Reiser

Summary

Water samples from Newman Lake and samples from a human female's mouth were collected. After plating and incubating the samples, four different bacterial colonies were selected for further investigation. Various biochemical and metabolic tests were performed on these isolates and later used in identification. The results for two of these unknowns were summarized and employed for identification. After analysis of the corresponding 16S sequences, it was determined that the unknown from the female mouth was most likely *Staphylococcus haemolyticus* or *S. xylosus*. The identity of the organism for the third unknown, which was from the aquatic environment, was determined to be either *Aeromonas veronii*, *A. calicicola*, or *A. jandaei*.

Introduction

The study of the microbial diversity found in the human body and the environment is both rewarding and beneficial. Changes in the populations or presence of various microorganisms can be both an indicator of new environmental factors and a new source for possible disease. The goal of the current study is to isolate and characterize different bacteria from two sources; one of these environmental and from the human body.

The human mouth was the first site selected for study. Organisms typically found in the mouth are usually not sensitive to light or a slightly acidic pH, can grow either aerobically or anaerobically,typically metabolize various sugars very well, and also can be incredibly resilient to antibiotics. The various genera that have been found in the human oral cavity are as follows: *Streptococcus, Lactobacillus, Fusobacterium, Veillonella, Corynebacterium, Neisseria, Actinomyces, Geotrichum, Candida, Capnocytophaga, Eikenella, Prevotella*, and several spirochetes(Madigan and Martinko 2006).

The water sample, was taken from a small creek that collects runoff water on the JMU campus. This creek is most likely heavily polluted due to fertilizer runoff and the presence of fecal matter from many local farm animals. Various species are found in water sources on the East Coast including *Protechacteria* (alpha- and beta-subdivisions), the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group, the *Actinobacteria*, the *Holophage* and the *Verrucomicrobia* (Zwart et al. 2002). Many different species of coliform bacteria, such as *Klebsiella spp. Enterobacter spp., Citrobacter spp.* may also be found in these environments, and are usually an indicator of water pollution.

Materials and Methods

Organism collection: freshwater aquatic environment

A water samples was carefully collected from the mouth of Newman Lake in 50-mL test tube at a depth of about 3-4" below the surface. Samples were vortexed and spun for 2 minutes at 13000xG. The pellet left in solution was inoculated onto tryptic soy agar (TSA) plates using the simple streak isolation technique (Leboffe & Pierce, 2006, p. 20-21). The plates were incubated for 24 hours at room temperature (RT).

Organism collection: oral cavity

Sterile cotton-tipped swabs dipped in phosphate buffer solution (PBS) were used for sample collection. The tongue of a female student was swabbed for 30 seconds. The swabs were then streaked onto TSA plates and incubated for 24 hours at 37° C.

Isolate testing

Sample testing was performed with standard procedures as described by Leboffe and Pierce (2006).

- Growth characteristics: pH, temperature, and thioglycollate.
- Growth rate: growth curve and serial dilutions(Temple 2007b).
- Fermentation capabilities: Methyl Red, Vogus-Proskauer and Triple Sugar Iron
- Respiration: catalase, oxidase and SIM tests
- Enzymatic activity: coagulase, urease, nitrate reduction and citrate utilization
- Selective and differential media: MSA, EMB, and blood agar
- Kirby-Bauer antibiotic resistance: penicillin, bacitracin, erythromycin, tetracycline, and sulfamethoxazole & trimethroprim.

PCR was performed as described by Temple (2007a) and samples sent for sequencing to be analyzed with RDP and BLAST. All media was prepared in the laboratory or purchased from an outside laboratory preparation company.

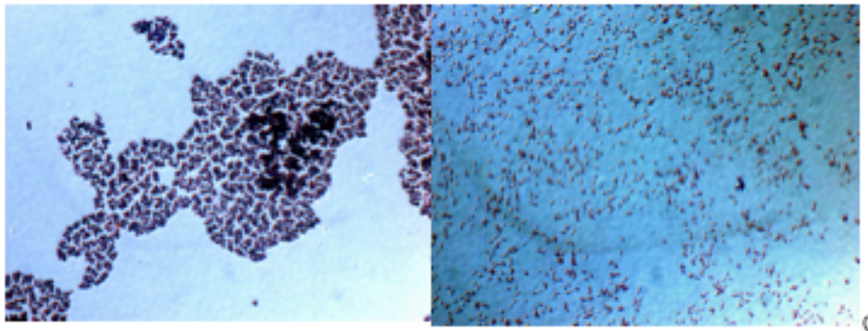


Figure 1. Gram stains of Unknown #1(left) and Unknown #3(right). Stains were digitally photographed under 1000x oil immersion microscope.

Table 1. Shows the results of biochemical tests for each unknown. Results that are highlighted indicate those that were important in identification of the suspected species. Unknown #1 was identified as either *Staphylococcus haemolyticus* or *S. xylosus*. Unknown #3 was identified as either *Aeromonas veronii*, *A.calicicola*, or *A. jandaei*.

Test	Unknown#1	Unknown #3
colony pigment	milky-white	white center transparent edges
Gram stain	(+) cocci	(-) rods
temperature	37° C	23° > 37° C
pH	7 > 8	7 > 8 > 5 > 3
aerobic growth	+	+
anaerobic growth	-	+
Methyl Red	-	+
V-P	-	inconclusive
glucose	+	+
glucose-gas	-	+
lactose/sucrose	+	+
sulfur reduction	-	-
indole production	-	-
catalase activity	+	+
oxidase activity	-	-
coagulase	-	Not Tested
urease	-	Not Tested
nitrate reduction	Not Tested	+
citrate utilization	Not Tested	+
MSA	+ (no acid)	Not Tested
EMB	Not Tested	+
hemolysis	β	-
motility	-	+

Table 2. Shows the results from BLAST analysis of 16S DNA sequencing. Red corresponds to Unknown #1 and blue corresponds to Unknown #3.

Organism	Max. Identity	E Value
S. haemolyticus	95%	4×10^{-128}
S. xylosus	93%	2×10^{-125}
A. veronii	98%	3×10^{-152}
A. calicicola	98%	1×10^{-151}
A. jandaei	98%	1×10^{-150}

Results

Colonies from Unknown #1 were punctiform and convex in appearance with smooth edges and a shiny, milky-white color. Staining revealed gram-positive cocci organized into irregular clusters (Figure 1). Colonies from Unknown #3 were small, round with smooth edges, and convex. They were shiny with an off-white center and transparent edges. Staining revealed gram-negative rods primarily as singlets and pairs joined by their termini (Figure 1). Results from growth in thioglycollate characterized Unknown #1 as possibly microaerophilic and Unknown #3 as a facultative anaerobe (Table 1).Results from various biochemical tests (Table 1) were also obtained and used for identification purposes in conjunction with 16S sequence analysis from Polymerase Chain Reaction (PCR) products. The 16S sequences were compared to known sequences using BLAST analysis. For Unknown #1, sequence analysis indicated a 95% maximum identity with *S.haemolyticus* and 93% identity with *S. xylosus* (Table 2). For Unknown #3, there was a 98% maximum identity with three organisms- *A. veronii*, *A. calicicola*, and *A. jandaei*(Table 2).

Discussion/Conclusions

The results of PCR analysis revealed that Unknown #1 was most likely *Staphylococcus haemolyticus* or *S. xylosus*. Based on colony morphology and staining results, either species could be present. Both are members of the *Staphylococcus* genus and are gram positive cocci. Growth at 37°C and at a pH between 7 and 8 also corresponds with the conditions from which the sample was isolated. Thioglycollate test results suggest a strictly anaerobic form of respiration, which is also consistent with this genus. Fermentation, SIM testing, and MSA plate results corresponded with characteristics of the genus as well. However, there was no acid produced on the MSA plate or coagulase present, which indicates that the strain is not *S. aureus*. Results from catalase and oxidase testing further supported *S. haemolyticus* or *S. xylosus* as the likely identity of the unknown.

The results of PCR analysis revealed that Unknown #3 was most likely *Aeromonas veronii*, *A. calicicola*, or *A. jandaei*. Gram stain results and cell shapes are consistent with *Aeromonas* . Similar support for this hypothesis came from thioglycollate testing which revealed that the organism is probably a facultative anaerobe. *Aeromonas* is a reasonable explanation for the unknown as it has been isolated previously from fresh water as well as sewage.

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