Investigation, Identification, and Classification of *Serratia marcescens*

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Hypothesis

My pet is *Serratia marcescens*.

Morphology

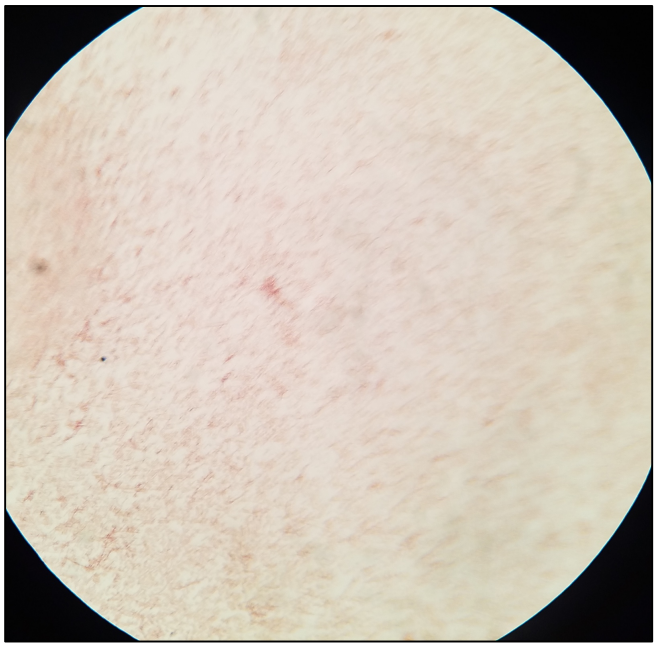

Figure 1. Gram stain of my pet, showing the retention of safranin, indicating a Gram negative bacteria.

My pet is a Gram negative bacteria. When the Gram staining procedure was performed on my pet, it was pink in color, as shown in Figure 1. My pet also grew on MacConkey and EMB agar, mediums which allow for the growth of Gram negative bacteria but prevent the growth of Gram positive bacteria.

My pet had medium-to-large round colonies with smooth edges and a dark, red, and shiny appearance. The cells of my pet were bacillus in shape, also shown in Figure 1.

Growth Media

Several growth media were used to test the ability of my pet microbe to grow in different environments. When incubated at 37 °C, my pet microbe grew on both NA and T-Soy agar. When plated on NA, it grew at room temperature (24 °C), 28 °C, and 37 °C. It did not grow at -20 °C, 4 °C, 45 °C, or 50 °C. My pets preferred growth medium was NA agar. Although it did grow on T-Soy, it grew more effectively on NA. When cultured on agar with different concentrations of salt, my pet grew at 0.85%, 3.5%, and 7.5% salt concentration. It did not grow at 15% salt concentration.

Anaerobic vs. Aerobic Growth

In order to test whether my pet can grow anaerobically, two plates were prepared. Each plate was trisected and streaked with my pet and two controls – *E. coli* as a positive control for growth in both environments, and *P. fluorescens* as a negative control for anaerobic growth and positive control for aerobic growth. One plate was incubated in an aerobic environment, while the other was incubated in an anaerobic environment. After incubation, the *E. coli* was the only bacteria to grow on the anaerobic plate, while all three bacteria grew on the aerobic plate. This shows that my pet is an aerobic bacterium.

Ability to Ferment Different Sugars

Several experiments were completed to investigate whether my pet microbe ferments several different sugars.

To test for the secretion of proteases, a skim milk agar was utilized. My pet, as well as two controls – *E. coli* as a negative control for the secretion of proteases, and *B. subtilis* as a

positive control for the secretion of proteases – were streaked on skim milk agar plates and stored at 28 °C for two days. Each control grew as expected. My pet had a clearing of skim milk around it, indicating that it does secrete proteases.

7.5% Mannitol agar was used to investigate whether my pet ferments mannitol and can withstand 7.5% sodium chloride concentration. Two controls were used – *S. epidermidis* as a negative control for the fermentation of mannitol, and *S. aureus* as a positive control for the fermentation of mannitol. After incubation for two days at 28 °C, both controls grew as expected, but my pet did not grow. Therefore, it is impossible to state whether my pet ferments mannitol or not.

MacConkey agar was used to test for Gram negative bacteria that ferment lactose. Four controls were utilized, as presented in Table 1.

	Fermentation of Lactose	**Growth**
E. coli	Strong fermenter	Yes (Gram negative)
E. aerogenes	Weak fermenter	Yes (Gram negative)
P. fluorescence	No fermentation	Yes (Gram negative)
B. subtilis	N/A (no growth)	No (Gram positive)

Table 1. Controls for MacConkey Agar Experiment.

These plates, along with my pet, were streaked and stored at 28 °C for two days. After the two days, the controls had grown as expected and my pet had also grown and had a dark purple color change, suggesting that my pet ferments lactose.

Eosin methylene blue (EMB) agar was utilized to test for the growth of Gram negative bacteria as well as the fermentation of lactose, similar to the MacConkey medium. Four controls were used, as shown in Table 2. These four controls and my pet were streaked on EMB agar and

stored at 28 °C for two days. My pet did grow on the EMB agar and had a purple color change, suggesting that it ferments lactose. The controls grew as predicted.

	Growth	Color Change	+/- Control for Growth	+/- Control for Color Change
E. Coli	Yes	Yes	+	+
E. aerogenes	Yes	Yes	+	+
P. fluorescens	Yes	No	+	-
B. subtilis	No	No	-	-

Table 2. Controls for EMB Agar Experiment

Durham tubes were used to determine if my pet produces acids and/or gases from three different sugars – lactose, glucose, and sucrose. Three controls were utilized – *E. coli, B. subtilis, and M. luteus.* The results of the experiment are presented in Table 3.

Table 3. Durham tube experiment controls and results.

	Sucrose	Glucose	Lactose
E. coli	Gas production No color change	Gas production Yellow color change	Gas production
B. subtilis	No gas production No color change	No gas production No color change	No gas production No color change
M. luteus	No gas production No color change	No gas production No color change	No gas production No color change
Pet	No gas production Yellow color change	No gas production Yellow color change	No gas production No color change

These results suggest that my pet does produce acid from sucrose and glucose, but none from lactose.

Motility

Two experiments were utilized to test for the motility of my pet.

Two bridge plates, each with nutrient agar on one side, water agar on the other, and filter paper connecting the two over the bridge, were used. One side of each paper was touching the water agar, with the other side touching the nutrient agar. *M. luteus* was used as a negative control for motility on one plate, while my pet was place on the other plates filter paper on the water side. After incubation, my pet showed growth on the nutrient agar side of the bridge plate, opposite of the side it was placed on. This suggests that my pet is motile and traveled from the water agar side of the plate to the nutrient agar side. The *M. luteus* control did not grow, as expected.

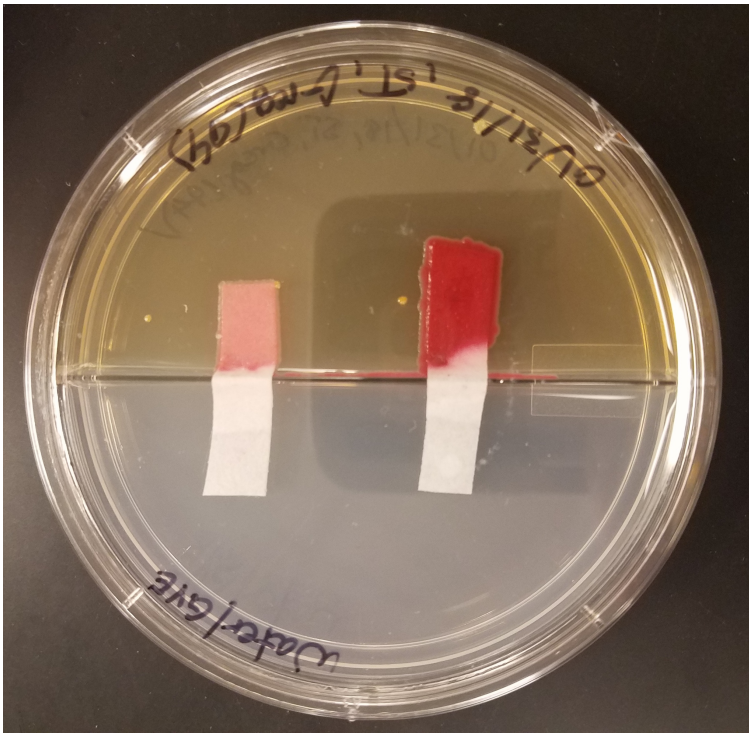

Figure 2. Bridge plate with water agar and nutrient agar, showing the motility of my pet from the water agar to the nutrient agar.

A swarm agar plate was also utilized to confirm that my pet is motile. My pet was placed in the middle of a swarm agar plate and incubated. Movement was seen, further supporting that my pet is motile.

Antibiotic Resistance

To test antibiotic resistance in my pet, several antibiotics that have different mechanisms of action were utilized, as presented in Table 4. Small paper disks containing the antibiotic were placed on plates covered in a thick layer of my pet. A control was utilized, which was a paper disk with no antibiotic on it. These plates were incubated at 28 °C for two days. The amount of clearing around the disk of antibiotic was used to quantify how effective the antibiotic was in stopping the growth of my pet. These results are presented in Table 5.

Antibiotic	Mechanism of Action
Erythromycin	Inhibition of protein synthesis
Chloramphenicol	Inhibition of protein synthesis
Doxycycline	Inhibition of protein synthesis
Tetracycline	Inhibition of protein synthesis
Ampicillin	Destruction of peptidoglycan in cell wall
Penicillin	Destruction of peptidoglycan in cell wall
Control	N/A

Table 4. Mechanisms of action of the antibiotics used.

Antibiotic	Diameter Effected
Erythromycin	3 cm
Chloramphenicol	3 cm
Doxycycline	3.5 cm
Tetracycline	2.5 cm
Ampicillin	0 cm
Penicillin	0 cm
Control	0 cm

Table 5. Effectiveness of antibiotics in terms of diameter.

The only antibiotics that effected my pet in a significant way were those targeting protein synthesis. None of the antibiotics that targeted the peptidoglycan in the periplasm were effective.

Effect of Pet Microbe on Fruit

When a grape was inoculated with my pet and incubated at 28 °C for two days, no bacterial growth was seen on the grape. This suggests that my pet is not a plant pathogen.

Classification of Pet Microbe

- Domain: Bacteria
- Phylum: Proteobacteria
- Class: Gamma Proteobacteria
- Order: Enterobacteriales

- Family: Enterobacteriaceae
- Genus: Serratia
- Species: *Serratia marcescens*

S. marcescens is a Gram negative, aerobic, bacillus, and motile bacteria, consistent with my pet (Herra et al., 2017). Red pigment, prominent in my pet, is a differentiating biotip of *S. marcescens.* Good growth at 5°C is uncommon in over 90% of strains, while growth at 40°C is common in over 90% of strains, consistent with my pet. *S. marcescens* also forms acid when it encounters sucrose, glucose, and lactose. One peculiarity in my pet is that the Durham tube experiment showed no indications of acid production when it interacted with lactose (Balows et al., 1991). *S. marcescens* uses proteases to break down peptide bonds in several compounds, which the skim milk experiment has shown my pet capable of accomplishing. All strains of *S. marcescens* are considered to be resistant to ampicillin and similar antibiotics, consistent with my pet's data (Auwaerter, 2007). *S. marcescens* is also quite susceptible to antibiotics such as doxycycline and chloramphenicol, consistent with my pet (Traub, 2001). This evidence supports the hypothesis that my pet is *S. marcescens.*

Other Students with *S. marcescens*

- Emily Sausman
- Taylor Bickel

References

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