



**BIOL 3200-005**  
**Unknown Lab Report**

---

**Unknown #545**

***Enterococcus faecalis***

---

Henry G. Norrell III (Manny)  
Lab: Tuesdays 3:00-4:50 pm  
2 December 2014

**INTRODUCTION:**

This experiment was performed to identify an unknown microorganism by applying numerous methods learned in previous laboratory assignments. Testing to identify microorganisms is applicable in all aspects of biological studies ranging from food production to medicinal studies. The methods and results from all tests used to identify the unknown are presented in this report. A hand written lab record sheet is also included at the end of this report.

**MATERIALS AND PROCEDURES:**

An inoculated slant labeled Unknown #545 was issued for identification. Initially a gram stain of the microorganism was made to determine its morphology and if it was gram positive or gram negative. A Brightfield Microscope with a final magnification of 1000, using a 100x objective and immersion oil, concluded the gram stain results. The unknown microorganism was determined to be gram positive cocci. The bacterium appeared in clusters and individually on the stain smear. A picture of the gram stain smear can be seen in Figure 1 below.

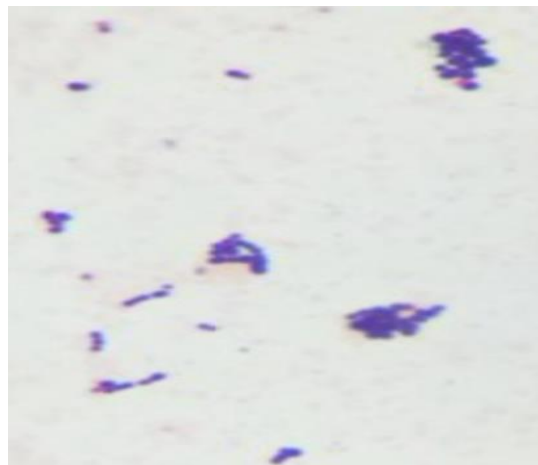


Figure 1: Gram Stain smear of Unknown #545 using 100x Objective

Two Trypticase Soy Agar (TSA) plates were streaked for colony isolation. These streaks were incubated at 37° C and used for further tests after twenty-four hours of growth. A Sheep Blood Agar (SBA) plate was also used to streak for isolation and incubated at 37°C for twenty-four hours. Each TSA plate produced moderate growth with white opaque colonies of the

unknown. The streaks showed a beaded path of round colonies along the lines of the streak. One of the TSA streak plates can be seen below in Figure 2. The unknown also showed moderate growth of identical colonies on the SBA plate. The growth on the SBA plate showed no hemolysis or discoloration, interpreted as gamma hemolysis. A picture of an isolated colony on the SBA plate is presented in Figure 3. Isolated colonies from these streaks were used in various biochemical tests to determine the genus and species of the unknown.

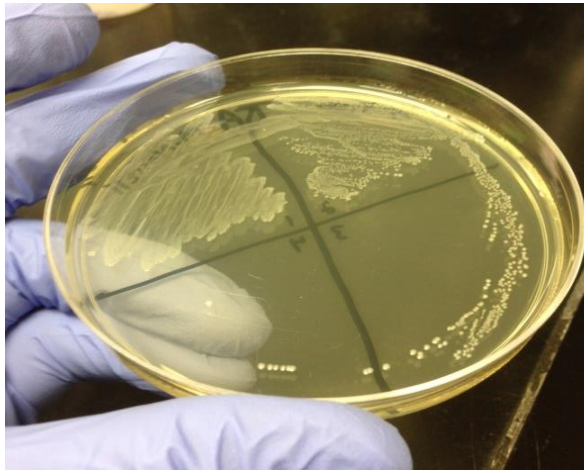


Figure 2: Isolated Colonies of Unknown #545 on TSA Streak Plate

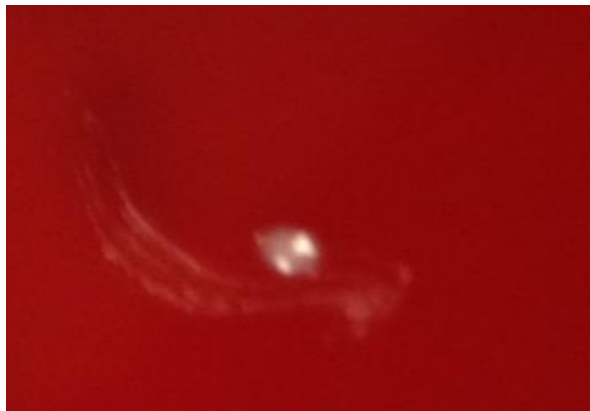


Figure 3: Isolated colony of Unknown #545 on SBA Plate

## RESULTS:

Table 1: Biochemical Tests




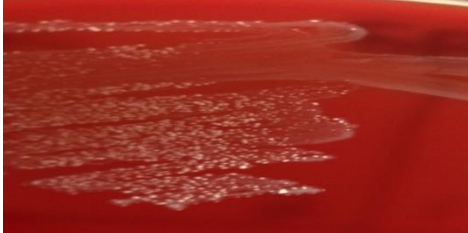




Test	Media/reagents	Enzyme or End Products Indicated by Test	Interpretation	Picture
Catalase	Slide, 3% $H_2O_2$	Enzyme test for Catalase enzyme production by organism.  End Products: $H_2O_2 \rightarrow H_2O + O_2$	Negative (No bubbles)	
Oxidase	Oxidase Reagent (N,N,N',N'-tetramethyl-p-phenylene-diamine)	Enzyme test for cytochrome C oxidase in bacteria.  End products: None	Negative (no color change)	
KOH	Slide, 3% KOH	Enzymes: none  End Products: Cell wall lysis and release of DNA	Negative (no strings)	
Hemolysis on SBA	Sheep Blood Agar plate	Tests for organism's hemolytic activity on SBA  End Products: none	Gamma ( $\gamma$ ) Hemolysis (non-hemolytic)	
Mannitol	Tube with 0.5-1% Mannitol and Phenol Red pH indicator	Enzyme test for enzyme(s) used in fermentation of Mannitol  End Products: Acid and Gas	Positive (yellow = fermentation)	
Bile Esculin Hydrolysis	Slant Agar with bile, sodium azide, and esculin	Test for hydrolysis of esculin  End Products: esculetin and dextrose	Positive	

Table 2: Additional Confirmation Tests

Test	Media/ reagents	Enzyme or End Products Indicated by Test	Interpretation	Picture
Optochin Susceptibility	SBA plate streaked with microorganism, Optichin disk	Differentiates based on susceptibility to ethyl hydrocupreine hydrochloride  End Products: none	Resistant	
BHI broth	Tube , 6.5 % NaCl	Differentiates microorganisms based on ability to grow in NaCl  End Products: Growth of microorganism in tube	Positive (Turbidity)	

**DISCUSSION:**

The first three biochemical tests determined that the unknown belonged to the *Streptococcaceae* family. The gamma hemolysis on the SBA plate also helped ascertain the microorganism was either *Enterococcus faecalis* or *Streptococcus agalactiae*. After a positive result from the Mannitol fermentation test it was decided that Unknown #545 was *Enterococcus faecalis*. It was the only possible member of the *Streptococcaceae* family that ferments Mannitol. All additional biochemical tests confirmed the identification of the unknown and no problems were encountered.

*Enterococcus faecalis* is a gram positive bacterium that inhabits the gastrointestinal tracts of humans or other mammals. *E. faecalis* also has a heat tolerance similar to more harmful bacterial strains that contaminate foods during processing. Because of this it is used as a surrogate in heat-processing tests designed to kill harmful food-borne pathogens (Kim, 2012). A Food Process Engineer could consider this information invaluable when developing and enhancing heat-processing protocols for certain foods.

**REFERENCE:**

Kim, E. B., L. M. Kopit, L. J. Harris, and M. L. Marco. 2012 "Draft Genome Sequence of the Quality Control Strain *Enterococcus Faecalis* ATCC 29212." *Journal of Bacteriology* 194.21

**Dichotomous Key To Find Unknown Organism:**

