

# Identification of a unknown bacterium essay sample



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Although bacteria is microscopic in size, it is largely important in the healthcare field, environmental work, food preparation, as well as many other industries. In particular, it is essential that healthcare workers be able to identify the species of bacteria invading a human reservoir in order to prescribe the correct antibiotic that will kill that species. For the purpose of bacteria identification, numerous tests have been devised to find out the exact species in question. However, because new strains continue to emerge, it is of the utmost importance that microbiologists and microbiology students understand the nature of each bacterial species and how that species creates and maintains its complex communities. Of equal importance is the quick identification of the contaminating source. The purpose of this study is to identify an unknown bacterium using as few tests as possible, and discover its importance in the world at large. Materials and Methods

To begin this study, a slant of bacteria was given by the instructor labeled “ unknown 1.” From this culture, three T-streak plates were aseptically inoculated and one was placed in a 37 degrees Celsius incubator, one was placed in a 25 degrees Celsius incubator (to check for optimal growing temperature, as well as purity of the culture), and the other was placed in a gas pak pouch. A reserve culture was also made which replaced the working slant after 21 days of use. After the cultures had incubated for 2 days and tested pure, a Gram stain was performed from the working slant, and an oxidase test was performed using a TSA plate. The gas pak system was used by creating an inoculated TSA plate (using aseptic techniques) and placing it in a GasPak pouch. A generating packet was placed inside the GasPak pouch

and the pellet attached to the pouch was observed for a white color, indicating that oxygen was absent. The GasPak was then placed in the 25 degrees Celsius incubator for 48 hours and observed for growth. The Gram stain was accomplished by preparing a smear and applying crystal violet to it, and allowing it react for 2 minutes.

The crystal violet was then washed off by squirting water onto one end of the slide, tilting it and allowing the excess violet color to wash off. The slide was then flooded with Gram's iodine and allowed to react for 1 minute, 30 seconds. The slide was then rinsed again with water using the same technique as before, however this time the excess water was shaken off. The next step was tilting the slide at an angle (hot dog style) and adding acetone-alcohol one drop at a time until the the color came off. It was then crucial to completely rinse the slide with water. After the slide was rinsed, it was time to add the safranin to the slide, to which it was allowed to react for 60 seconds. The excess safranin was then tilted off the slide and washed with water. The slide was then allowed to dry. The oxidase test was performed to find out whether the unknown bacteria had cytochrome oxidase in its electron transport chain. This was achieved using the TSA plate from the 37 degrees Celsius incubator and streaking the bacteria onto an oxidase card using a toothpick. The card was then observed for a blue color within a 20 second time frame. The next test performed was fermentation of lactose.

In order to test for lactose fermentation, a tube of phenol red lactose broth was inoculated from the working slant and then incubated at 37 degrees Celsius for 48 hours. After incubation, the phenol red lactose broth was  
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examined for color change. The fifth test performed was to find out if the unknown bacteria could utilize citrate as its sole source of carbon. This was done using an inoculating needle and aseptically transferring the bacteria into a slant of Simmon's citrate agar by stabbing the needle into the butt of the agar, then streaking it across the top of the agar as the needle was pulled out. The tube was then placed in the 37 degrees Celsius incubator for 48 hours, observed for a blue color, then placed back in the incubator for another 5 days and observed again. The sixth and seventh tests performed was the fermentation of sucrose and arabinose. This was performed by aseptically inoculating a tube of phenol red sucrose broth, and a tube of phenol red arabinose broth with the unknown culture and incubating at 37 degrees Celsius for 48 hours. After incubation, the two tubes were examined for color change. The eighth test was to find out if the bacteria in question had flagella.

The motility test was performed by aseptically inoculating the unknown bacteria into a tube of TSA broth and allowing it to incubate for 24 hours at 37 degrees Celsius. After the TSA broth culture had incubated, it was used to inoculate a tube of motility medium S to the third. The inoculated motility medium was then incubated at 37 degrees Celsius for another 24 hours and observed for red streaks radiating from the stab line. The ninth test was intended to find out if the unknown bacteria can produce acetoin which is the precursor to butanediol. The Voges-Proskauer test was performed by aseptically inoculating a tube of MR-VP broth with the unknown bacteria and incubating it at 37 degrees Celsius for 5 days. The incubated tubes were examined for growth. After examinations were recorded, 1 ml of the

inoculated MR-VP broth was transferred to a clean test tube and then one ampule of reagent A was added. The mixture was then lightly shaken for 30 seconds before adding 5 drops of reagent B. This mixture was then aerated vigorously for 30 seconds and then allowed to sit for 10 minutes, but was aerated frequently during that time.

After the 10 minutes was up, the tube was observed for a pink color (pink indicates a positive test). The tenth test was to find out if the unknown bacteria had the ability to produce cysteine desulfurase, which produces hydrogen sulfide from the amino acid cysteine. This test was performed by using a Kligler iron agar slant and an inoculating needle to aseptically stab a culture of the unknown bacteria into the butt of the slant, then streaking it across the top of the agar as the needle is pulled out. The inoculated Kligler iron agar slant is then incubated at 37 degrees Celsius for 48 hours. The tube is then observed for blackening of the agar (indicating a positive test). The eleventh test was to find out if the unknown bacteria contained the enzyme tryptophanase, which gives the bacteria the ability to hydrolyze tryptophan into indole, pyruvic acid and ammonia.

The indole test was performed when a tube of tryptone broth was aseptically inoculated with the unknown and incubated for 24 hours. After the tube had incubated, 1 dropper full of Kovac's reagent was added to the tube. The tube was then aerated and observed for a red layer (indicating a positive test).

The final tests were to determine whether the bacteria could ferment dulcitol and melibiose. The test was performed by obtaining a tube with a pellet containing dulcitol and phenol red and another tube with a pellet containing melibiose and phenol red (these are called a wee-tab). 0.25ml of sterile <https://assignbuster.com/identification-of-a-unknown-bacterium-essay-sample/>

water was then added to each tube and then vibrated until the pellets dissolved in the water. Both wee-tabs were then inoculated with a thick loopful of the unknown bacteria and incubated at 37 degrees Celsius for 30 minutes, observed, then incubated for another 5 hours 30 minutes and observed again for a color change. Results

Unknown 1 had the following morphology on a TSA plate: medium sized, smooth, circular colonies that were off-white in color. After determining that it was a Gram negative small rod that was a facultative anaerobe, an oxidase test was performed to verify the family of the bacteria. The oxidase test was negative, showing that Unknown 1 belonged to the family Enterobacteriaceae. Table 1 lists all the tests that were performed, along with the results.

## Discussion

To discover the family of the bacteria “ Unknown 1”, a Gram stain was performed. Under 1000X magnification, the bacteria was pink and rod shaped meaning it was a gram negative, bacillus bacteria. This eliminated all the Gram positive bacteria as well as all the cocci bacteria which allowed for specific testing. After the discovery that the unknown bacteria was a gram negative rod, it was important to find out if it was aerobic or facultative. The gas pak system resulted in a positive test by showing minimal growth on the TSA plate. This indicated that the unknown bacteria was a facultative anaerobe, meaning it uses and prefers aerobic respiration, but can also switch to fermentation when oxygen is not present. In order to find out whether Unknown 1 belonged to the family Enterobacteriaceae,

Vibrionaceae or Pasteurellaceae, an oxidase test was performed. A negative  
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oxidase test indicated that the bacteria could not use cytochrome oxidase in its electron transport chain. Because of the negative Gram staining reaction and a negative oxidase test, it became known that this bacteria belonged to the family Enterobacteriaceae. In an environment where oxygen is not present, a facultative anaerobe will ferment sugar to produce ATP.

Because the phenol red lactose broth remained red, there was no acid production to cause the indicator to change from red to yellow, indicating a negative test. The fermentation of sucrose and arabinose were also tested in the same way, however the acid production caused by the bacteria breaking down these two sugars resulted in both tubes turning yellow, indicating positive tests. Based on these tests all bacteria that were able to ferment lactose were crossed off the possible species list, as well as those that could not ferment sucrose and arabinose. If a bacterium possesses the enzyme citrase, it will be able to use citrate as its sole source of carbon. The bacteria in question produced a positive citrate utilization test. The positive test indicated that this species of bacteria contains citrase and can convert citrate to pyruvate and carbon dioxide.

All bacteria that did not contain citrase were crossed off the list of possible bacteria species. Flagella are too small to see using a light microscope, so a motility medium was used in order to see whether this bacterial species can move around. The red streaks radiating from the stab line indicated that the bacterium has flagella. Because the bacteria was so well spread throughout the medium, it can be considered very motile. Based on the motility test, all bacteria species that were non-motile were crossed off the identification list.

The Voges-Proskauer test is intended to identify the pathway in which a  
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microorganism ferments glucose. If the bacterium produces butanediol as an end product, it undergoes butanediol fermentation. Because Unknown 1 tested negative for this test, it must ferment glucose by mixed acid fermentation. All bacteria that could complete butanediol fermentation were eliminated from possible species of bacteria. Certain species of bacteria will produce hydrogen sulfide from the amino acid cysteine. This unknown species of bacteria tested positive for hydrogen sulfide production, indicating it is capable of catabolizing cysteine.

This is important in certain environments when cysteine can be used as an energy source for respiration. Any bacteria that could not use cysteine as an energy source were eliminated. The indole production test was used to find out if this species of bacteria could hydrolyze tryptophan into indole, pyruvic acid and ammonia. The red layer at the top of the test tube showed a positive test for the unknown species of bacteria indicating that it possess the enzyme tryptophanase. All bacterial species that did not contain this enzyme were eliminated. Dulcitol fermentation and melibiose fermentation were the final tests used to distinguish between the bacteria that could ferment these sugars and those that could not. The red colored dulcitol wee tab indicated a negative test, while the yellow melibiose wee tab indicated a positive test. This final test showed that Unknown 1 was *Citrobacter freundii*. Below is a flow chart that lists all the bacteria that were eliminated with each test.