Activity 5.1.5: Student Resource Sheet

Biochemical tests are the most definitive way to identify bacterial species. Each biochemical test helps determine a property or characteristic specific to a certain bacterial species. These tests determine which growth media the bacteria will grow on and identify the end products of their metabolic processes, such as the wastes they excrete. For example, in one biochemical test, the bacterial sample is inoculated in a growth media containing a specific nutrient that only certain bacterial samples are able to metabolize, or break down. The media also contains an indicator. If the bacterial sample metabolizes the nutrient, it excretes acid as a waste product, causing the indicator to change color, indicating a positive result. Many tests often need to be performed in order to positively identify a bacterium. Laboratories have developed shortcuts that allow scientists to perform several biochemical tests at one time. Below is a description of each of the biochemical tests performed on Anna's bacterial sample.

Ornithine Decarboxylase Test: Test to determine if the microbe can use the amino acid ornithine as a food source. The microbe is incubated in ornithine decarboxylase broth. The microbe must first use the glucose present to cause the pH to drop, which causes the color to change from purple to yellow. Once the medium has been acidified, the enzyme ornithine decarboxylase is activated. The culture is incubated an additional 24 hours at 35-37 C to allow the microbe to now use the ornithine. The final results are then observed at 48 hours. Change back to purple from yellow indicates a positive test for ornithine decarboxylase. Failure to turn yellow at 24 hours or to revert back to purple at 48 hours indicates a negative result.

Citrate Utilization Test: Test to determine if the microbe can use citrate as its sole source of food. It is often used to differentiate between members of *Enterobacteriaceae*. If an organism is capable of utilizing citrate as a carbon source, CO_2 is produced. If CO_2 is produced, it reacts causing the pH indicator (bromthymol blue) in the media to turn from green to blue.

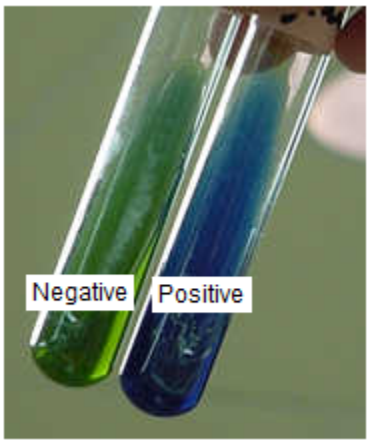
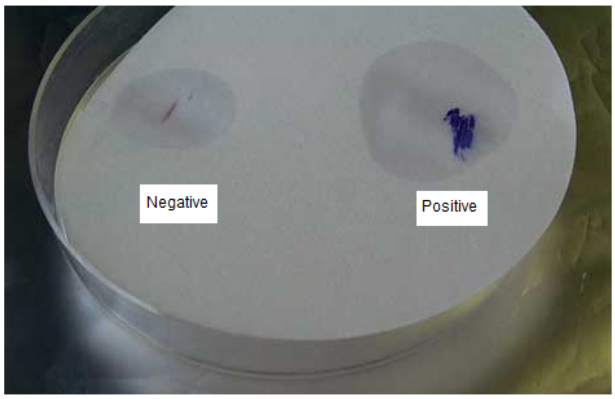


Image obtained from **http://www.uwyo.edu/molb2210_lab/info/biochemical_tests.htm**

Voges-Proskauer Test (VP Test): Test to determine if the microbe produces acetoin as a fermentation product from glucose. If the culture is positive for acetoin, it will turn brownish-red to pink. If the culture is negative for acetoin, it will turn brownish-green to yellow.

Image obtained from **http://www.uwyo.edu/molb2210_lab/info/biochemical_tests.htm**

Oxidase: Test to identify microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). It is commonly used to distinguish between oxidase negative Enterobacteriaceae and oxidase positive Pseudomadaceae. In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns a dark purple.



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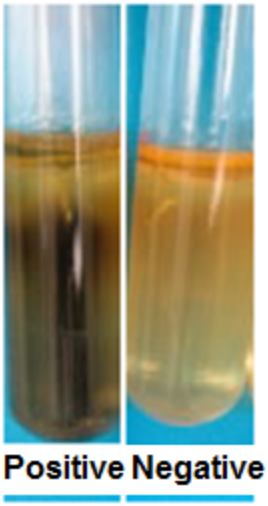
Lysine Decarboxylase Test: Test to determine if the microbe can use the amino acid lysine as a food source. The microbe is incubated in lysine decarboxylase broth. The microbe must first use the glucose present to cause the pH to drop, which causes the color to change from purple to yellow. Once the medium has been acidified, the enzyme lysine decarboxylase is activated. The culture is incubated an additional 24 hours at 35-37 C to allow the microbe to now use the lysine. The final results are then observed at 48 hours. Change back to purple from yellow indicates a positive test for lysine decarboxylase. Failure to turn yellow at 24 hours or to revert back to purple at 48 hours indicates a negative result.

H2S: Test to determine if the microbe reduces sulfur-containing compounds to sulfide during metabolism. If sulfide is produced, it combines with iron compounds to produce FeS, a black precipitate. Several media containing iron compounds allow detection of hydrogen sulfide production. One medium used is Sulfide-Indole-Motility

(SIM) medium. This is a nutrient medium allowing the detection of three different traits in bacteria and contains sulfates to serve as the substrate for detecting sulfide production.



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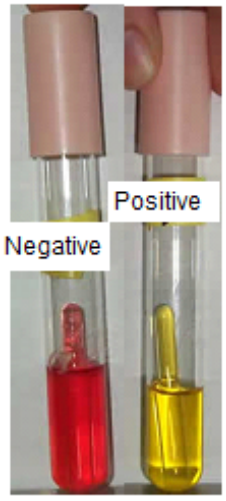
Glucose Fermentation Test: Test to determine if the microbe can ferment the sugar glucose. The microbe is grown in a broth that contains glucose and the pH indicator, phenol red. If an organism is capable of fermenting the sugar glucose, then acidic byproducts are formed and the pH indicator turns yellow.



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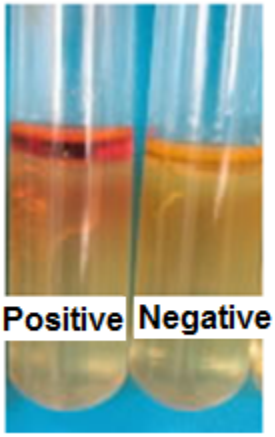
Lactose Fermentation Test: Test to determine if the microbe can ferment the sugar lactose as a food source. The microbe is incubated in phenol red lactose broth for 24 hours. If the microbe ferments lactose, the media become acidic, causing a color change from red to yellow.

Indole Test: Test to determine if the microbe produces indole as a byproduct from the metabolism of the amino acid tryptophan. If indole is produced, it will react with a chemical reagent added after incubation to produce a color change. There are two

media that are used for this test: Sulfide-Indole-Motility (SIM) medium and Tryptone broth medium. In SIM media, indole reacts with added Kovac's reagent to form rosindole dye which is red in color



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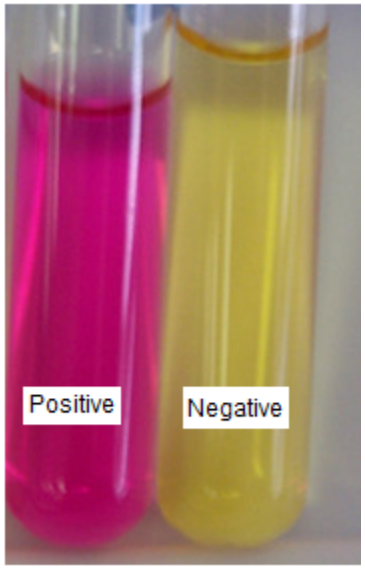
Urease Test: Test to identify bacteria capable of hydrolyzing urea using the enzyme urease. The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media and the pH indicator, phenol red, turns from yellow to pink.



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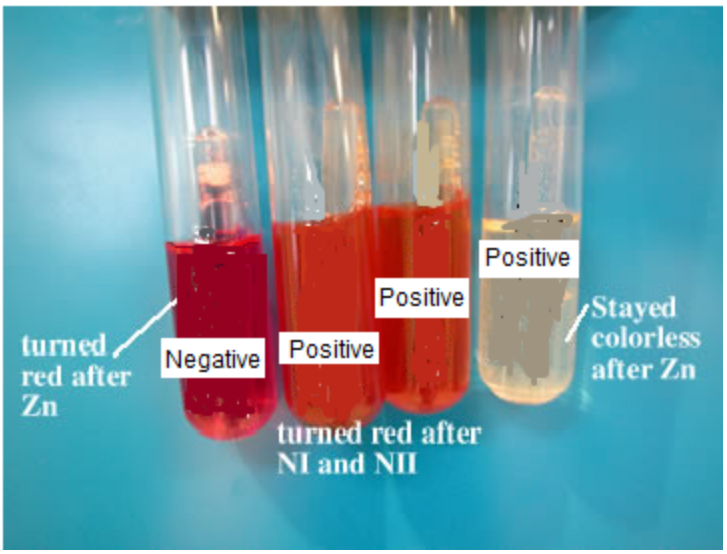
Nitrate Reduction Test: Test to determine if an organism is capable of reducing nitrate to nitrite or other nitrogenous compounds via the action of the enzyme nitratase. If nitrite is present in the media, it will react with nitrate I and nitrate II to form a red compound (a positive result). If no red color forms, zinc is added to the broth to convert any remaining nitrate to nitrite. If no color change occurs upon the addition of zinc, then this means that the nitrate was converted to some other undetectable form of nitrogen (a positive result).



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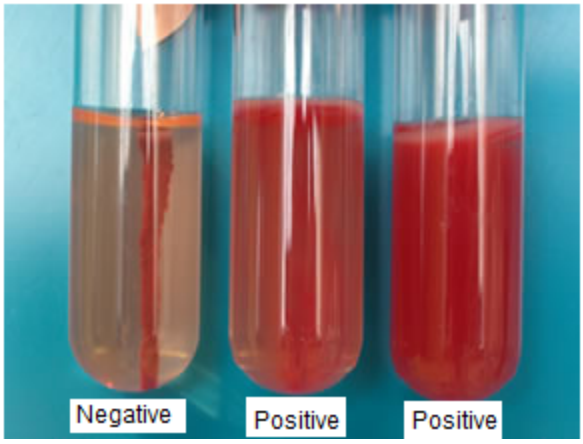
Arabinose Test: Test to determine if the microbe can ferment the sugar arabinose as a food source. The microbe is incubated in phenol red arabinose broth for 24 hours. If the microbe ferments arabinose, the media become acidic, causing a color change from red to yellow.

Motility Test: Test to determine whether an organism is equipped with flagella and thus capable of swimming away from a stab mark. If the entire tube is turbid, or cloudy, this indicates that the bacteria have moved away from the stab mark and are motile. If the stab mark is clearly visible and the tube is not turbid, the organism is likely non-motile.



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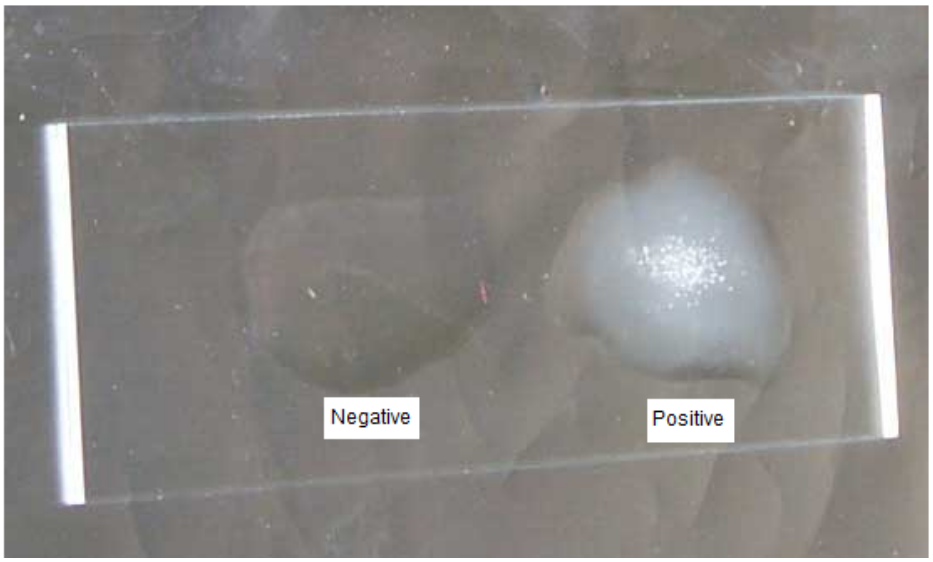
Gelatin Hydrolysis: Test to determine if the microbe can use the protein gelatin as a source of carbon and energy for growth. The microbe is incubated in nutrient gelatin. If the microbe uses gelatin, the medium changes from semisolid to liquid and cannot be resolidified.

Catalase Test: Test to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. The bubbles resulting from production of oxygen gas clearly indicate a catalase positive result.



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