Distinguishing Species of Bacteria

Mike Wetoszka
UW ID: 20394714
Partner: Justin Hoffmann
TA: Terin Robinson and Jason Tan
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Introduction

The purpose of this experiment is to distinguish species of bacteria by cultivating in different media and doing some tests. Another purpose of this lab is to learn the proper techniques of testing for fermentation of carbohydrates, production of indole, activity of urease, production of hydrogen sulfide, evidence of amylase activity, evidence of lipase activity, and evidence of protease activity.

Different species of bacteria can be distinguished on the basis of the carbohydrates they do or do not utilize, as well as the nature of the products formed in the fermentation reaction. (Madigan and Thomas, 2009) Bacteria are able to ferment or breakdown simple carbohydrates to produce acidic, alcoholic, or gaseous end products. (Goldman 2009) By testing which bacteria species will ferment which carbohydrate and what products are formed allows one to identify. (Madigan and Thomas, 2009) The medium for the test is a nutrient broth with the acid-base indicator bromocresol purple. The test tube contains a Durham tube to collect gas that will be released in the medium when the carbohydrate is fermented. (Goldman 2009) Some organisms will not ferment at all, some will produce acid products and no gas, and some will make both acid products and gas. (Davidson, 2010) After inoculation, the tubes are incubated at 37ºC which is the optimal temperature for growth. A negative result for the carbohydrate test is no change in pH(no colour change) or gas production. (Goldman 2009) A positive result is gas produced and a acid production (yellow). (Goldman 2009) The second test is the indole test. Indole is a by-product of the metabolic breakdown of the amino acid tryptophan by the enzyme tryptophanase. (Davidson, 2010) Another by-product of this degradation is pyruvate which is used as an energy source for the bacteria. (Madigan and Thomas, 2009) The bacteria are grown on a medium

containing tryptophan and the presence of indole is detected by adding the chemical indicator, Kovac's reagent. The active ingredient in Kovac's reagent is p-dimethylaminobenzaldehyde, which reacts with indole to produce a deep pink colour. The negative result for this reaction is no colur change which means there is no indole. A positive for the indole test is a red/ pink colour. (Goldman 2009) The third carbohydrate metabolism test is the urease test. Proteus bacteria can split the urea molecule releasing carbon dioxide and ammonia. This reaction, mediated by the enzyme urease, can be seen if the culture medium is prepared with urea added as the substrate. (Davidson, 2010) Phenol red is also added as a pH indicator. When bacteria which generate urease are grown in this medium, degradation of urea with the release of ammonia can be detected as the pH becomes basic and the pH indicator color becomes reddish pink. (Goldman 2009) Therefore the positive result is a pink colour.

The first test of experiment 19 is the evidence of carbohydrase (amylase) activity. Another name for this test is the starch hydrolysis test. The test is used to find the presence of alpha-amylase. Alpha-amylase is an extracellular enzyme that acts on the 1,4-α-glucosidic bonds of starch. (Davidson, 2010) The two polysaccharides present in the starch polymer are amylose and amylopectin. Amylose absorbs iodine to produce a blue compound, while amylopectin produces a red-violet compound. (Madigan and Thomas, 2009) The starch hydrolysis test is performed by inoculating streaks of bacteria on starch agar plate. After incubation, the plate is then flooded with Gram's iodine solution. (Davidson, 2010) The presence of a colourless zone which is referred to as a halo surrounding the streaks of growth are an indication of a positive result which means starch hydrolysis took place. (Goldman 2009) The second test is the evidence of lipase activity. Hydrolysis of a lipid leads to the formation of glycerol and fatty acids. (Goldman 2009) The streak plate will appear cloudy or turbid. If the formation of the precipitate

is seen the test is positive for lipolysis. (Davidson, 2010) The third test is the evidence of protease activity. Casein is a nitrogenous protein found in the milk agar. (Madigan and Thomas, 2009) If a clear zone is surrounding the bacterial streak then the test is positive and the casein is hydrolyzed. The next test is the catalase test. Catalase is an endoenzyme. (Madigan and Thomas, 2009) Catalase acts on hydrogen peroxide, formed as an oxidative end product of aerobic respiration, catabolizing it into water and oxygen. (Madigan and Thomas, 2009) When bubbles of oxygen are observed when a hydrogen peroxide is added to a bacterial colony, the bacterial are considered catalase-producing microorganism. (Davidson, 2010) Another endoenzyme is oxidase. In the oxidase test, a drop of tetramethyl-p-phenylenediamine reagent is placed on a piece of filter paper. Using a toothpick rub a small amount of bacterial growth from a culture plate into the drop and observe for the development of a purple colour. (Goldman 2009) The purple colour should form rapidly in about 10 seconds for the test to be declared positive. If the colour change happens slowly or takes more than a minute, a negative result should be recorded.

Escherichia coli is an facultive anaerobic, Gram negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. (Madigan and Thomas, 2009) Pseudomonas aeruginosa is a common Gram- negative, aerobic, rod-shaped bacterium which can cause disease in animals. (Madigan and Thomas, 2009) Bacillus subtilis is a Gram-positive, catalase-positive bacterium commonly found in soil. Proteus vulgaris is a rod-shaped, Gram negative bacterium that inhabits the intestinal tracts of humans and animals. (Madigan and Thomas, 2009)

Materials and Methods

The protocol and materials used for the experiment were completed as written in the Department of Biology of the University of Waterloo Fall term 2010 Biology 140L Lab Manual. Experiment 18, Some Metabolic Activities of Bacteria, is found on pages 70 to 74. Experiment 19, Bacterial Enzymes, is found on pages 75 to 78. The only change made to the procedure is that the recording of urea broth tubes was not done on the urea broth tubes we prepared previously.

Results

Table 1: Carbohydrate Fermentation Test Results

Name of organism	Glucose	Lactose	Sucrose
Escherichia coli	AG	AG	N-
Pseudomonas aeruginosa	N-	N-	N-
Bacillus subtilis	A-	N-	A-
Proteus vulgaris	AG	N-	N-

Legend

A	Acid Production (yellow)
K	Alkaline Color Change (dark blue)
N	Neutral (purple)
G	Gas formation

The table above shows the results taken from the carbohydrate test. The colour and the formation of gas were observed in these tests. According to these results the Escherichia coli utilizes glucose, and lactose for fermentation. The Pseudomonas does not use none of the carbohydrates tested.

Table 2: Ideal Results for Carbohydrate Fermentation

Name of organism	Glucose	Lactose	Sucrose
Escherichia coli	AG	AG	N-
Pseudomonas aeruginosa	A-	A-	K-
Bacillus subtilis	A-	N-	A-
Proteus vulgaris	AG	N-	N-

The table above shows the ideal results for the carbohydrate test. As you can see the only difference with Table 1 are the results for Pseudomonas aeruginosa.

Table 3: Indole Production Test Results

Name of Organism	Colour of surface ring	Indole production
Escherichia coli	Red	+
Pseudomonas aeruginosa	None	-
Bacillus subtilis	None	-
Proteus vulgaris	None	-

The table above shows the results of the indole test. As the drops of Kovac's reagent mixed with the surface of the broths, the only bacteria which changed colours was Escherichia coli.

Table 4: Ideal Results for Indole Production

Name of Organism	Colour of surface ring	Indole production
Escherichia coli	Red	+
Pseudomonas aeruginosa	None	-
Bacillus subtilis	None	-
Proteus vulgaris	None	-

The table above shows that the ideal results match the results we have gotten in the lab which are recorded in table 3.

Table 5: Urease Activity Test Results

Name of Organism	Colour of broth before incubation	Colour of broth after incubation	Urease
Escherichia coli	Yellow	Yellow	-
Pseudomonas aeruginosa	Yellow	Yellow	-
Bacillus subtilis	Yellow	Yellow	-
Proteus vulgaris	Yellow	Pink	+

The table above shows the results for the urease activity. The observations were made on broths which we did not prepare. All of the crothes appeared yellow before and after incubation, expect for the proteus vulgaris which changed colour to pink.

Table 6: Ideal Results for Urease Activity

Name of Organism	Urease
Escherichia coli	-
Pseudomonas aeruginosa	-
Bacillus subtilis	-
Proteus vulgaris	+

The table above shows that the results for the urease from table 5 match the ideal result. Therefore no errors were made in that test.

Table 7: Production of Hydrogen Sulfide Test Results

Name of organism	Colour of SIM medium	Hydrogen Sulfide Production
Escherichia coli	Yellow/ Brown	-
Pseudomonas aeruginosa	Brown	-
Bacillus subtilis	Yellow/Brown	-
Proteus vulgaris	Black/Brown	+

The table above shows the results of the hydrogen sulfide production test. The only positive result is for the proteus vulgaris which showed a blackening of the medium.

Table 8: Ideal Results for Production of Hydrogen Sulfide

Name of organism	Hydrogen Sulfide Production
Escherichia coli	-
Pseudomonas aeruginosa	-
Bacillus subtilis	-
Proteus vulgaris	+

The table above shows that the results presented in table 7 match the ideal results.

Table 9: Exoenzyme Activity Test Results

Name of organism	Starch Hydrolysis	Lipolysis	Proteolysis
Escherichia coli	-	-	-
Bacillus subtilis	+	+	-
Pseudomonas aeruginosa	-	+	+

The table above shows the results for the starch hydrolysis test, lipolysis test, and the proteolysis test.

Table 10: Ideal Results for Exoenzyme Tests

Name of organism	Starch Hydrolysis	Lipolysis	Proteolysis
Escherichia coli	-	-	-
Bacillus subtilis	+	+	-
Pseudomonas aeruginosa	-	+	+

The table above shows that the results for the exoenyme tests are the same as the results from table 9.

Table 11: Endoenzyme Activity Test Results

Name of organism	Catalase	Oxidase
Escherichia coli	+	-
Bacillus subtilis	+	-
Pseudomonas aeruginosa	+	+

The table above shows that the results from the endoenyme activity results.

Table 12: Ideal Result of Endoenzyme Activity Test Results

Name of organism	Catalase	Oxidase
Escherichia coli	+	-
Bacillus subtilis	+	-
Pseudomonas aeruginosa	+	+

The table above shows that the ideal results are the same as the results from table 11.

Discussion

According to Table , Escherichia coli and Proteus vulgaris showed fermentative activity. However Proteus vulgaris only showed it for glucose, while E.coli showed it for all three of the carbohydrates. Pseudomonas aeruginosa showed no fermentation. In table 3, the only bacteria which showed a production of indole was Escherichia coli. This is because E. Coli has the enzyme tryptophanase that can degrade the amino acid tryptophan into indole, pyruvic acid and ammonia. (Madigan and Thomas, 2009)The idole produced by the bacteria binded with the p-dimethlyaminobenzaldehyde in the reagent to produce the red compound. (Madigan and Thomas, 2009) In table 5, the Proteus vulagris was the only positive bacteria for the urease test. This means that Proteus vulagris is a rapid urease-positive organism. The restrictive amount of nutrients coupled with the use of pH buffers prevent all but rapid urease-positive organisms from producing enough ammonia to turn the phenol red pink. (Madigan and Thomas, 2009) The Urease broth can be used to differentiate members of the genus Proteus. In table 7, the proteus

vulgaris is the only positive bacteria. The reason for this result is that Proteus Vulgaris is positive in the hydrogen sulfide test because it can undergo anaerobic respiration using Sulfur as an electron acceptor. (Madigan and Thomas, 2009)In table 9, Bacillus subtilis showed positive result on the starch hydrolysis whereas Escherichia coli did not. Starch hydrolysis requires the presence of exoenzyme amylase to hydrolyze starch into smaller polysaccharides. (Madigan and Thomas, 2009) Positive result of starch hyrdrolysis showed the ability of the mircoorgism to produce amylase. In table 11, showed that all of the bacteria are capable of producing catalase. Hydrogen peroxide is an superoxide which is able to be degraded by catalase. All of the bacteria where able to produce the enzyme to degrade it. In the oxidase test only Pseudomonas aeruginosa had a positive result. This is because it is the only microorganism out of the others which has oxidase. This means it takes part in cytochrome oxidase activity. Oxidase activates the oxidation of reduced cytochrome c by molecular oxygen in aerobic organisms during electron transport. (Madigan and Thomas, 2009) Oxidized cytochrome c transfers molecular oxygen to tetramethyl-p-phenylenediamine when the reagent is added to growth of an oxidase positive organism. (Madigan and Thomas, 2009)

This experiment has the possibility of human error. Mistakes could have been made by failing to sterilize the inoculating loop correctly, which would result in possible contamination of the sample. Another error could have been possibly occurred by mislabeling the plates according to species, which would produce invalid results.

In conclusion the experiment was successful. I learned to distinguish species of bacteria by cultivating in different media and doing some tests. I also practised the proper techniques of testing for fermentation of carbohydrates, production of indole, activity of urease, production of

hydrogen sulfide, evidence of amylase activity, evidence of lipase activity, and evidence of protease activity.

References

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