

LAB 4
BIOCHEMICAL PROPERTIES OF BACTERIA
AND DETECTION OF MOTILITY

Objectives

- In this lab you will learn how to:
 - test the ability of your unknown (UK) to digest certain substrates
 - discern motility of bacteria

Introduction

Various species of bacteria can digest, or **hydrolyze**, a wide diversity of carbohydrates, including sucrose, lactose, glucose, starch, mannose, xylose, cellulose, and chitin. Furthermore, bacteria often hydrolyze proteins, such as those in gelatin and milk.

Often, bacteria undergo **fermentation**, in which oxygen is not the final electron acceptor in the metabolic pathways that extract energy from the substrates. In addition, some microbes produce **acid** (such as lactic acid, acetic acid, and propionic acid) and **gas** (such as CO₂, CH₄, and H₂) as byproducts of hydrolysis.

In this part of the lab you will test the biochemical metabolic properties of your unknown bacteria.

Part I: Biochemical Tests

Obtain:

- carbohydrate fermentation tubes of:
 - glucose
 - lactose
 - sucrose
- tube of litmus milk medium
- tube of nutrient gelatin
- starch agar deep (melted)
- hydrogen peroxide
- your unknown bacteria (on nutrient agar slant)

Procedures

First, perform a **Gram stain** on your unknown bacteria, which are growing in the agar slant you prepared last week.

Are the colony and cell morphology descriptions the same as you recorded in Tables 1 and 2?

If so, continue.... If not, ask your instructor how to proceed.

1. Pour the melted starch agar tube into a new, sterile Petri dish and allow it to cool while you work on the other tubes, as follows.

After the starch agar plate has cooled, streak a single line of your UK down the center of the plate. Be sure to label the plate.

2. When you collect the glucose, sucrose, and lactose fermentation tubes, label them immediately! (It is easy to confuse the three tubes, all of which are filled with red broth.) Collect the other tubes of culture media and keep them upright in a rack.

Sterilize a transfer loop and obtain a very small quantity of your bacteria from the agar slant. Remember, just touching the surface of the agar is enough! Inoculate one tube at a time, then re-sterilize the loop before proceeding to the next tube.

Notice the small, inverted **Durham tube** in each carbohydrate fermentation tube; this will catch any gas if it is produced during fermentation.

3. Next, inoculate a tube of litmus milk medium with your UK, using the same aseptic technique.

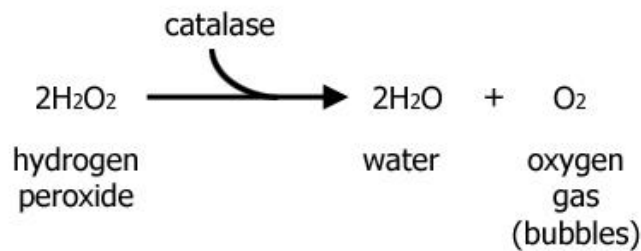
4. Also, inoculate a tube of gelatin agar with your UK, using aseptic techniques. Only this time, you will “**stab**” inoculate the sample down into the gelled medium in the tube.

Place all inoculated tubes in a rack in the incubator, and incubate at 37°C for 24-48 hours. Also, incubate the starch plates in an inverted position!

5. Finally, transfer some of your UK bacteria onto a slide and add a drop of hydrogen peroxide. Examine it closely for tiny bubbles.

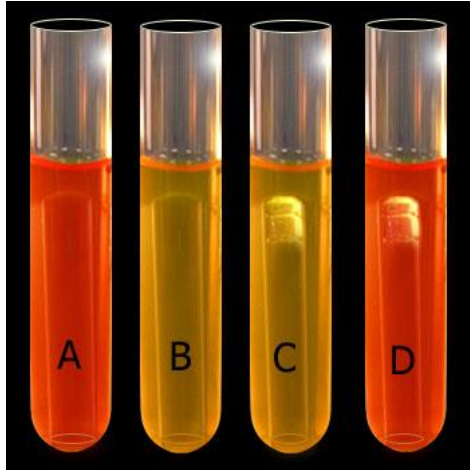
If your sample has bubbles, it is positive for the enzyme **catalase**. If there are no bubbles, it is negative for catalase.

Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen:



Observations

For each inoculated **carbohydrate fermentation** tube, record acid production results (red = no acid produced; yellow = acid produced) and gas production results (in the Durham tube). Compare your tubes to the following photo:



Carbohydrate fermentation results:

- A = no acid, no gas;**
- B = acid but no gas;**
- C = acid and gas;**
- D = no acid but gas**

For the inoculated **litmus milk** tube, record the appearance of the medium (more than one description may apply). Some examples of these results are shown in the photo, below.

Pink = acid reaction (lactose fermented with acid production)

Purple = lactose not fermented

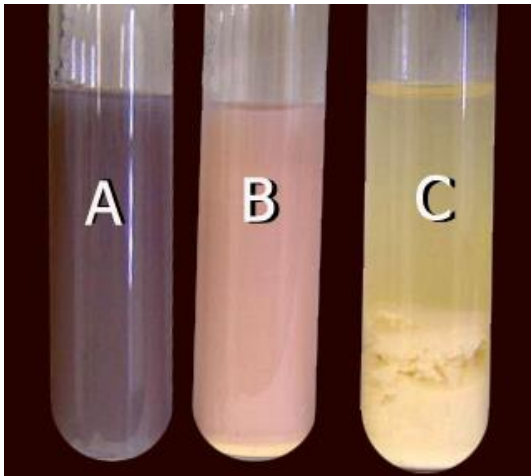
Blue = alkaline reaction (lactose not fermented; bacteria degraded nitrogenous materials in milk)

White = reduction of litmus by enzyme reductase

Clots or curds = milk protein coagulation

Clearing of medium = whey formation (peptonization; milk protein digested)

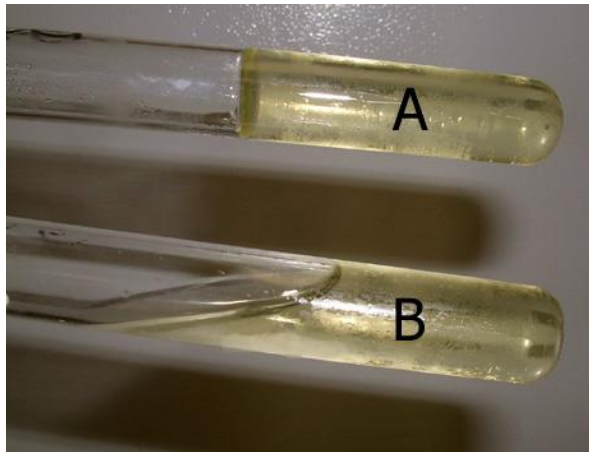
Bubbles or cracks in curds = gas formation (CO_2 and/or H_2)



Litmus milk medium results:

- A = no fermentation;**
- B = acid reaction (lactose fermented);**
- C = curd formation (protein coagulation),**
clearing of medium (whey formation), and
cracks in curd (gas formation).

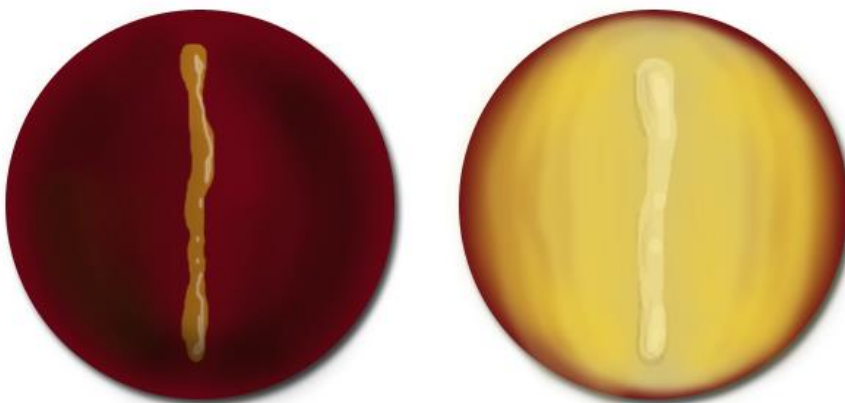
For the inoculated **nutrient gelatin** tube, record liquefaction (**positive for protein digestion**) or no liquefaction (**negative for protein digestion**) in the chilled tube. Was the gelatin completely or partially liquefied? Compare your tube to the following photo:



Protein (nutrient gelatin) hydrolysis results:

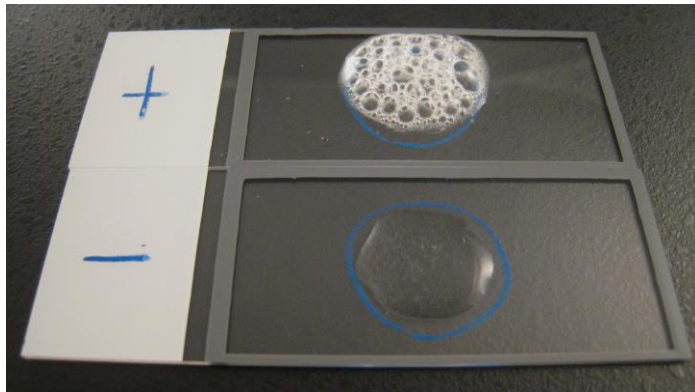
**A = no hydrolysis;
B = hydrolysis.**

For the inoculated **starch agar** Petri plate, cover the surface of the agar with iodine and let it sit for 1 minute. Starch turns dark black when it reacts with iodine, thus, if the colony is surrounded by dark-stained agar, it is **negative for starch digestion**. If the colony has a yellow halo around it, the starch is broken down and it is **positive for starch digestion**. Compare your plate to the following photo:



Starch hydrolysis results:

**Left = no hydrolysis;
Right = hydrolysis**



Catalase results:

**Top = positive for catalase;
Bottom = negative for catalase**

Fill out Table 1 with the biochemical properties of your UK sample. Include the biochemical test results and any other descriptions you think are important.

Table 3. Biochemical properties of your unknown bacteria.

BIOCHEMICAL TEST	RESULTS / DESCRIPTIONS
Glucose fermentation	
Sucrose fermentation	
Lactose fermentation	
Litmus milk reaction(s)	
Protein (gelatin) hydrolysis	
Starch hydrolysis	
Catalase test	

Part II: Additional Biochemical Tests

Enterotube II System

The **Enterotube II System** is a “rapid identification test system” that provides results for 15 different biochemical tests that are run simultaneously. The tests are conducted in a single compact unit (see photo below) that is divided into 12 compartments, each holding a different type of test medium. For example, the first compartment holds a glucose medium similar to that in a glucose fermentation tube, and it will change from red to yellow if acid is present.



To inoculate the Enterotube II System, a colony is first grown on a Petri plate. After removing a cap at one end of the unit, the exposed tip of an inoculating wire (which is already sterile) is used to pick up some bacteria from the colony on the Petri plate. The wire is then withdrawn through all 12 compartments, by pulling on it. The wire is then pushed back through the 12 compartments, further inoculating the media. The entire unit is placed in the incubator and any color changes after 24 hours are noted.

Using a chart provided with the Enterotube II System, any color changes after incubation are translated into a 5-digit number. Several test results are combined to create each number in the final 5-digit number. For example, if the glucose test appears yellow with gas production (seen as gas bubbles trapped in the compartment), it is assigned the number **3**. If it appears yellow with no gas, it is assigned the number **2**. If it is red with no gas, it is assigned the number **0**. By looking up the final 5-digit number in the Enterotube II System handbook, the species identification can be determined. For example, the number **34363** would identify the bacteria as *Klebsiella pneumoniae*.

The Enterotube II System is used to identify species of the **Family Enterobacteriaceae**, a group commonly referred to as the enterics. Enterics are bacteria that live inside the gastrointestinal tracts of animals, and are often responsible for cases of food poisoning. For example, using this test system, *Salmonella* can be quickly and easily discerned from *E. coli*. Other rapid identification test systems are available for identifying non-enteric species, such as *Staphylococcus* and other bacteria.

Part III: Detection of Motility

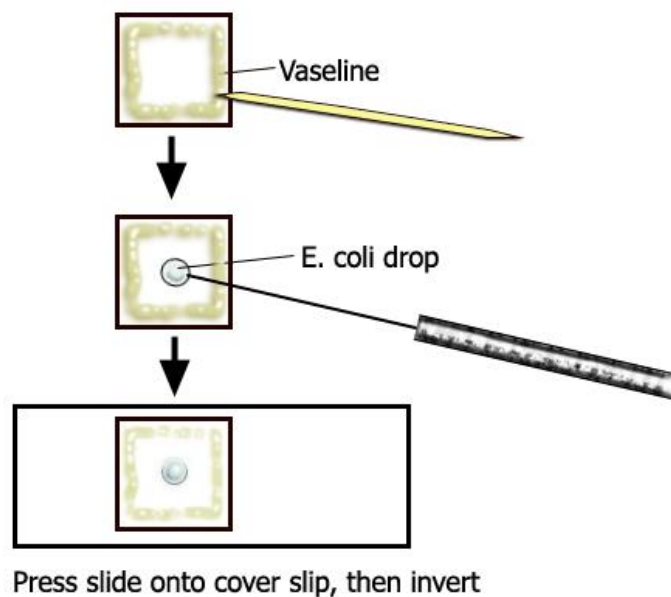
Some species of bacteria are able to move about via whip-like structures called **flagella**. The ability to move is referred to as **motility**. Motility allows bacterial cells to move towards nutrients or away from harmful substances.

The arrangement of flagella on the bacterial cell can be very important when identifying bacteria. For example, some bacteria have a single flagellum at one end (**monotrichous**), or one flagellum at each end (**amphitrichous**), or a tuft of flagella at one end (**lophotrichous**), or are covered in flagella (**peritrichous**). *E. coli*, for instance, is a motile bacterium that is peritrichous.

Flagella must be specially stained in order to be visualized using typical light microscopy. However, motility in living bacteria can be determined without staining, by using the **hanging drop preparation**, in which a drop of liquid media containing live bacteria is suspended from the coverslip and bacterial motion is observed.

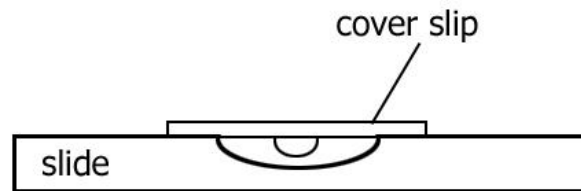
Procedures

To prepare a hanging drop slide of *E. coli*, clean a depression slide and feel for the depression with your finger. Along the edges of a clean coverslip, apply a tiny amount of Vaseline using a toothpick. Using aseptic techniques, transfer one or two loopfuls of the bacterial broth culture to the center of the coverslip. **DO NOT** flip the coverslip over! Instead, pick up the depression slide, flip it over and press it against the Vaseline-lined coverslip, so that the depression covers it without the slide touching the drop.



Preparing a hanging drop preparation of *E. coli*.

Invert the slide so that it looks like the following illustration, then examine the bacteria for motility under the microscope, as described below.



Observe live *E. coli* under careful control of illumination, as described below and as shown by the instructor.

To find the bacteria, focus at low power on the edge of the drop of broth culture. The drop should appear as a transparent, black-outlined “bubble” in the center of the coverslip. Increase the magnification to 400x, with proper lighting of the slide. Lighting is critical; the iris diaphragm must be almost closed and very dark to see the tiny bacteria (light colored) swimming around the edge of the drop. If you have difficulty finding the bacteria, ask your instructor for help.