

Lab 4. Metabolism and Clinical Testing.

Read Leboffe pp. 107, 111-112, 122-123, 127, 131-133, 153-155.

Bacteria conduct a wide variety of metabolism. Even closely related organisms that appear identical under the microscope may differ greatly in which molecules they can process and consume. Consumption of food molecules requires specific operons encoding genes for transport and catabolism, such as the *lac* operon for lactose. But food consumption also depends upon environmental factors. For example, at neutral or alkaline pH (high pH), glucose fermentation is favored because it acidifies the environment, whereas at acid pH (low pH) glucose fermentation is inhibited but decarboxylation of amino acids is favored for production of alkaline amines. The presence of specific food molecules, and specific environmental factors such as pH and temperature, determine which species will grow best.

Metabolic diversity has played key roles in the development of clinical and diagnostic microbiology. If a bacterial isolate from a patient is implicated in disease, it is critical to identify the organism. Identification is based on chemical tests that depend upon (1) **selective media**, the ability of an organism to grow in a given growth medium; (2) **differential tests**, the effects of metabolic processes on the growth medium, based on a color change in an indicator molecule.

In Lab 4, we will practice the use of selective media and of differential tests to distinguish potential pathogens. Warning: Please treat all organisms used this week as potential causative agents of disease. Use the highest standard of sterile technique.

1. Phenylethyl alcohol agar (PA agar). PA agar is a selective medium that permits growth of gram-positive bacteria, such as those that cause impetigo or strep throat. The molecule phenylethyl alcohol, however, inhibits or greatly slows the growth of gram-negative bacteria. Thus, PA agar can be used to screen a growth sample for gram-positives only; or to test whether an isolated strain is gram-positive.

Obtain two PA agar plates. Divide in quarters with a marker, and label the quarters. Streak a different species (of the four provided) on each quarter. Perform in duplicate (two plates in all). Incubate overnight at 37°C. Observe again after three more days (Monday).

2. Lactose MacConkey agar (Mac agar). Mac agar is a selective medium that permits growth of gram-negative bacteria, particularly enteric bacteria (Enterobacteraceae) such as *E. coli* that grow in your intestines. The medium contains bile salts, which inhibit growth of most gram-positive bacteria. Mac agar is used to selectively isolate gram-negative species.

Note that Mac agar is also a differential test, in that *lac* positive strains (containing the *lac* operon) produce a red color due to acidification and shift in the color spectrum of an indicator. Mac agar can also be used with sugars other than lactose. For example, sorbitol Mac agar is used as an inexpensive first-line test for *E. coli* O157-H7, a serious pathogen from contaminated beef and manure.

Obtain two Lactose Mac agar plates. Divide in quarters with a marker, and label the quarters. Streak a different species (of the four provided) on each quarter. Perform in duplicate (two plates in all). Incubate overnight at 37°C.

3. Phenol red broth tubes. Phenol red medium is a differential test for sugar fermentation. The phenol red indicator turns yellow upon acidification. Besides broth suspension, phenol red tubes generally include 1% sugar of a given type; we will use glucose, lactose, and sucrose (each in isolation) plus a control tube lacking sugar.

Note that each tube also contains an inverted Durham tube. The Durham tube fills with gases produced by some forms of fermentation. Thus, phenol red tubes act as a differential test for gas-producing fermentation.

Obtain 4 phenol red tubes. Add 1 ml of 10% sugar (glucose, lactose, or sucrose) to each tube, except the control. Inoculate with one species of enteric bacteria. Incubate overnight at 37°C. Observe growth and color.

4. Decarboxylase tubes. Amino acids are decarboxylated by certain enzymes, as a part of catabolism for food, and in response to acidic pH conditions. Acid in the environment induced decarboxylation of amino acids, leading to production of amines, which alkalinize the environment. For example, lysine decarboxylase generates cadaverine, and arginine decarboxylase generates agmatine. The presence of lysine decarboxylase is typical of *E. coli*, whereas absence of lysine decarboxylase would be typical of *Shigella flexneri* and other enteric pathogens. In decarboxylase medium, the dye bromocresol purple turns purple upon alkalization.

Obtain 3 decarboxylase tubes. Add 1 ml of 10% lysine or arginine to each tube, except the control. Inoculate with one species of enteric bacteria. Incubate at 37°C. Observe growth and color the next day, and again after three days total.

Bacteria tested

Enterococcus faecalis

Escherichia coli

Proteus mirabilis – Use separate plate.

Pseudomonas aeruginosa

Salmonella enterica

Discussion points

1. Discuss the advantages and limitations of each test. If a test did not work as presented, explain why it might not, and how the method might be improved.
2. Why might a given test work for one strain but not for another? Why might a test pose problems for certain strains?
3. Using on-line sources, find a couple of examples of diagnostic use of the tests you performed. What clinical conditions were diagnosed using your tests? What results were obtained?
4. Using a microbial genome database, identify for each test one specific gene and protein (enzyme) responsible for the test result. Databases used may include NCBI, KEGG, and Ecogene.
5. Show the specific chemical reactions, with structures, involved in sugar fermentation and in amino acid decarboxylases, as used in your tests.

