

# **Fermentation**

#### **Objectives**

After completing this exercise, you should be able to:

- 1. Define fermentation.
- 2. Perform and interpret carbohydrate fermentation tests.
- 3. Perform and interpret the MR and V-P tests.
- 4. Perform and interpret the citrate test.

#### **Background**

Once a bacterium has been determined to be fermentative by the OF test, further tests can determine which carbohydrates, in addition to glucose, are fermented; in some instances, the end-products can also be determined. Many carbohydrates—including monosaccharides such as glucose, disaccharides like sucrose, and

polysaccharides such as starch—can be fermented. Many bacteria produce organic acids (for example, lactic acid) and hydrogen and carbon dioxide gases from carbohydrate fermentation (Figure 14.1). A fermentation tube is used to detect acid and gas production from carbohydrates. The fermentation medium contains peptone, an acid-base indicator (phenol red), an inverted tube to trap gas, and 0.5-1.0% of the desired carbohydrate. In Figure 14.2, the phenol red indicator is red (neutral) in an uninoculated fermentation tube; fermentation that results in acid production will turn the indicator yellow (pH of 6.8 or below). When gas is produced during fermentation, some will be trapped in the inverted, or Durham, tube. Fermentation occurs with or without oxygen present; however, during prolonged incubation periods (greater than 24 hours), many bacteria will begin growing oxidatively on the peptone after exhausting the carbohydrate supplied, causing



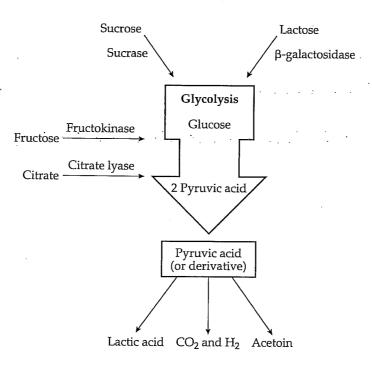


Figure 14.1



Fermentation. Bacteria are often identified by their enzymes. These enzymes can be detected by observing a bacterium's ability to grow on specific compounds. For example, *E. coli* and *Salmonella* are distinguished because *E. coli* can ferment lactose, and typical *Salmonella* cannot.

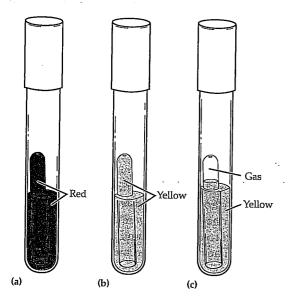


Figure 14.2

Carbohydrate fermentation tube. (a) The phenol red indicator is red in a neutral or alkaline solution. (b) Phenol red turns yellow in the presence of acids. (c) Gases are trapped in the inverted tube while the indicator shows the production of acid.

neutralization of the indicator and turning it red due to ammonia production.

Fermentation processes can produce a variety of end-products, depending on the substrate, the incubation, and the organism. In some instances, large amounts of acid may be produced, while in others, a majority of neutral products may result (Figure 14.3a). The MRVP test is used to distinguish between organisms that produce large amounts of acid from glucose and those that produce the neutral product acetoin. MRVP medium is a glucose-supplemented nutrient broth used for the methyl red (MR) test and the Voges-Proskauer (V-P) test. If an organism produces a large amount of organic acid from glucose, the medium will remain red when methyl red is added in a positive MR test, indicating that the pH is below 4.4. If neutral products are produced, methyl red will turn yellow, indicating a pH above 6.0. The production of acetoin is detected by the addition of potassium hydroxide and  $\alpha$ -naphthol. If acetoin is present, the upper part of the medium will turn red; a negative V-P test will turn the medium light brown. The chemical process is shown in Figure 14.3b. The production of acetoin is dependent on the length of incubation, as shown in Figure 14.4.

The ability of some bacteria to ferment citrate can be useful for identifying bacteria. When citric acid or sodium citrate is in solution, it loses a proton or Na<sup>+</sup>

**Table 14.1**Simmons Citrate Agar

Ingredient	Amount
Sodium citrate	0.2%
Sodium chloride	0.5%
Monoammonium phosphate	0.1%
Dipotassium phosphate	0.1%
Magnesium sulfate	0.02%
Agar	1.5%
Bromthymol blue	0.0008%

6

Qi.

to form a citrate ion. Bacteria with the enzyme citrate lyase can break down citrate to form pyruvate, which can be reduced in fermentation. Simmons citrate agar (Table 14.1) contains citrate as the only carbon source and ammonium  $(NH_4^+)$  as the only nitrogen source. When bacteria use citrate and ammonium, the medium is alkalized because of ammonia  $(NH_3)$  produced from  $NH_4^+$ . The indicator bromthymol blue changes to blue when the medium is alkalized, indicating a positive citrate utilization test.

#### **Materials**

Glucose fermentation tube Lactose fermentation tube

Sucrose fermentation tube

MRVP broths (4)

Simmons citrate agar slants (2)

#### Second Period

MRVP broth

Glucose fermentation tube

Simmons citrate agar slant

Parafilm squares

Empty test tube

Methyl red

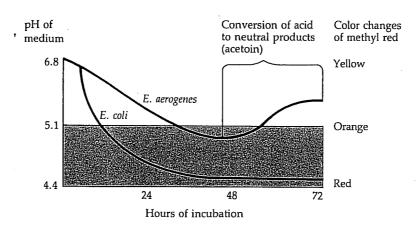
V-P reagent I, α-naphthol solution

V-P reagent II, potassium hydroxide (40%)

(b) 
$$\begin{array}{c} CH_3 \\ C=O \\ H-C-OH + \\ CH_3 \\ CH_3 \end{array} + KOH + O_2 \longrightarrow \begin{array}{c} OH \\ C=O \\ C=O \\ CH_3 \\ CH_3 \end{array} + COndensation \\ CH_3 \\ Acetoin \\ Acetoin \\ Acetoin \\ CAPACHUMA \\ Acetoin \\ (catalyst) \end{array}$$

Figure 14.3

MRVP test. (a) Organic acids, such as lactic acid, or neutral products, such as acetoin, may result from fermentation. (b) Potassium hydroxide (KOH) and  $\alpha$ -naphthol are used to detect acetoin.



**Figure 14.4**. The production of acetoin is dependent on incubation time and pH.

## **Cultures (as assigned)**

Escherichia coli

Enterobacter aerogenes

Alcaligenes faecalis

Proteus vulgaris

## **Techniques Required**

Inoculating loop technique, Exercise 10

Aseptic technique, Exercise 10

OF test, Exercise 13

#### **Procedure**

#### Fermentation Tubes

- 1. Use a loop to inoculate the fermentation tubes with the assigned bacterial culture.
- 2. Incubate the tubes at 35°C. Examine them at 24 and 48 hours for growth, acid, and gas production. Compare them to an uninoculated fermentation tube. Why is it important to record the presence of growth?
- 3. Record your results with this culture, as well as your results for the other species tested. (See Color Plate III.2.)

#### **MRVP Tests**

1. Using a loop, inoculate two MRVP tubes with Escherichia and two with Enterobacter.

2. Incubate the tubes at 35°C for 48 hours or longer. Why is time of incubation important (Figure 14.4)?

1

1

- 3. To one tube of each set (Escherichia and Enterobacter), add 5 drops of methyl red. Record the resulting color. Red indicates a positive methyl red test (Figure 14.3a).
- 4. To the other set of two tubes, add 0.6 ml (12 drops) of V-P reagent I and 0.2 ml (2 or 3 drops) of V-P reagent II.
- Cover each tube with a Parafilm square, and shake the tubes carefully. Discard the Parafilm in the disinfectant jar.
- 6. Leave the caps off to expose the media to oxygen in order to oxidize the acetoin (Figure 14.3b). Allow the tubes to stand for 15 to 30 minutes. A positive V-P test will develop a pinkish-red color.
- 7. Create controls. Pour half of the contents of an uninoculated MRVP broth into an empty test tube. Perform the MR test (step 3) on one tube. Perform the V–P test (steps 4–6) on the other tube.
- 8. Record your results. (See Color Plates III.3 and III.4.)

#### Citrate Test

- 1. Using a loop, inoculate one citrate slant with Escherichia coli and the other slant with Enterobacter aerogenes.
- 2. Incubate the tubes at 35°C until the next lab period.
- 3. Compare the tubes to an uninoculated citrate slant and record your results. (See Color Plate III.5.)

# Exercise 14

# **Fermentation**

_	and the second		
ADADAG	DOTE	Drnor	<b>~~</b>
I A KORAT	ITHE	KEPIN	<i>?</i> ' F '
LABORAT	LOKI		L

Name	
Date	
LAB SECTION	Ν

## Data

6

(()

## Fermentation Tubes

Color of uninoculated medium:

						(	Carbohy	drate					
			Glucos	е			Lactos	se			Sucros	se	
Organism		Growth	Color	Acid	Gas	Growth-	Color	Acid	Gas	Growth	Color	Acid	Gas
Escherichia coli	24 hr												
	48 hr												
Enterobacter aerogenes	24 hr				. 1		,						
	48 hr												
Alcaligenes faecalis	24 hr												
	48 hr	7											
Proteus vulgaris	24 hr												
	48 hr												,

## **MRVP Tests**

		N	1R	V	P <sub>.</sub>
Organism	Growth	Color	+ or -	Color	+ or -
Escherichia coli					
Enterobacter aerogenes					
Controls					

6

# Citrate Test

Organism	Growth	Color	+ or -
Escherichia coli			
Enterobacter aerogenes			
Controls			

# Questions

	What would happen if an organism used up all the carbohydrate in a fermentation tube?
	What would the organism use for energy? What color would the indicator be then
2.	If an organism metabolizes glucose aerobically, what result will occur in the fermentation tubes?

# **Critical Thinking**

- 1. Could an organism be both MR and  $V\!-\!P$  positive? Explain.
- 2. Could an organism be a fermenter and also be both MR and V-P negative? Explain.
- 3. How could you determine whether a bacterium fermented the following carbohydrates: mannitol, sorbitol, adonitol, or arabinose?
- 4. If a bacterium cannot ferment glucose, why not test its ability to ferment other carbohydrates?