

## Lab 13: Bacteria of the Digestive System

### Learning Objectives

- Identify how selective and differential media can be used in the identification of bacteria that can inhabit the digestive tract.
- Identify the growth of samples of presumed fecal bacteria from rest room swabs.

### Introduction

The human intestinal tract is inhabited from birth by a variety of microorganisms acquired, at first, from the mother. Later, microbes are ingested in food and water, and from hands and objects placed in the mouth. Many of those do not survive the acidic pH of the stomach, which helps prevent infection from pathogens. However, those that do survive find the environment in the small intestine and colon more hospitable. Generally, our normal intestinal microbiota are harmless and are beneficial in two ways: they outcompete potential pathogens for space and nutrients; and produce vitamins, including vitamin B<sub>12</sub>, folic acid, biotin, and vitamin K, that we cannot synthesize ourselves.

Most of the intestinal bacteria are anaerobic. However, when feces are cultured, facultative anaerobic bacterial species can be observed. These normal inhabitants of the intestinal tract are Gram-negative, non-spore forming bacilli. They grow rapidly and well under aerobic conditions on most nutrient media. The use of selective and differential culture media allows their isolation and identification.

In this experiment three bacteria that are often part of the normal intestinal microbiota, *Escherichia coli*, *Enterobacter aerogenes*, and *Serratia marcescens* will be investigated using selective and differential media, and carbohydrate fermentation.

Selective media typically contain substances that either favor the growth of certain microorganisms or inhibits the growth of others. Differential media are formulated so that the growth of particular organisms will result in a change in the color of the media or a change in the color of the bacterial colonies.

**MacConkey agar (MAC)** is both a selective and differential medium. Crystal violet and bile salts inhibit the growth of Gram-positive microorganisms, so only Gram-negative bacteria will grow. This medium contains lactose as its sugar source. Lactose fermenters will metabolize this sugar and secrete lactic acid as a waste product of lactose fermentation. This causes a localized drop in the pH around the colony, which, in turn, causes a pH indicator, neutral red, in the agar to turn bright pink. Colonies also appear pink. Non-lactose fermenters cannot utilize lactose and therefore do not cause a change in the pH. These colonies appear white on MacConkey agar, and the agar plate background turns yellow from the basic waste products normally produced by cells. The colonies of weak lactose fermenters may be pink, but the agar is yellow.