Exercise 25
BACTERIOLOGICAL EXAMINATION OF WATER

Introduction

The use of ***Escherichia coli*** as an **indicator** of water-borne pathogens was first suggested in the early 1890s. This organism type was proposed because it was found to be uniformly present in the intestinal tracts of humans and other animals, and its presence outside was regarded as being due to contamination with intestinal discharges.

The Bacteriological examination of water (or food) is conducted to detect the presence of bacteria that are indicators of contamination by excrement or decomposing plant material. These bacteria, including *Escherichia, Klebsiella, Citrobacter* and *Enterobacter* species (among others) are called **coliform** bacteria because they are normally associated with the **colon** or large intestine. They are all Gram-negative bacilli (rods) that belong to the family **Enterbacteriaceae**.

Bacteria of the genera *Enterobacter* (formerly *Aerobacter*), *Klebsiella* and *Citrobacter* are widely distributed, free-living and parasitic forms, often found in large numbers in decaying organic material. Members of the genus *Escherichia* are quite similar, but are entirely parasitic/mutualistic, occurring in large numbers in the alimentary tracts of humans and other animals. Although many strains of *Escherichia coli* are not normally pathogenic, some can cause infection of the bladder and kidney if they gain access to those organs, and also cause peritonitis when the intestine is punctured and the contents escape into the peritoneal cavity. Some strains of *Escherichia coli* have recently been shown to cause severe gastrointestinal infection, dysentery, hemolytic-uremic syndrome and even death in susceptible individuals.

The above genera are distinguished from other intestinal parasites in the family Enterobacteriaceae by their ability to ferment lactose with the production of acid and gas. This capacity is not wide spread among bacteria, and thus an anaerobic medium containing lactose is convenient for detecting the presence of these forms. This fact, coupled with the occurrence of *Escherichia* in great numbers in the alimentary canal has led to the widespread use of lactose containing broths for detecting fecal contamination of food and drinking water.

Important pathogens of the genus *Salmonella* (etiological agents of enteritis and typhoid), and *Shigella* (agents of bacillary dysentery), also live in the alimentary tracts of diseased as well as carrier animals. They do not ordinarily occur in such numbers that it is feasible to examine water and food directly for them, and it is not particularly significant if water or food being subjected to pollution appears to lack them. If pollution from fecal material exists, there is always the possibility of infection by these dangerous pathogens as well as others such as *Giardia* and hepatitis B viruses. *Escherichia coli* provide a more sensitive index to potential infection than do the more serious pathogens, since these bacteria are more abundant and widespread.

Standard Methods

The "**Standard Methods**" tests; for the examination of water and food for coliform bacteria includes a series of steps known as the **presumptive**, **confirmatory**, and **completed** tests. These are typically carried out in sequence over several days, and although various specific media preparations may be used, the general format remains consistent as described below.

Presumptive Test

In the presumptive test a sample of the water being examined is inoculated into a fermentation tube containing lactose and other nutrients needed to support growth (various, specific preparations have been developed, some double-strength broths and some dry). A small inverted tube (Durham tube) inside the larger tube serves to trap any gas produced by the fermentation of the lactose. Gas formation within 48 hours of incubation at 37^{o} C constitutes a positive presumptive test. If a pH indicator is present in the medium, a color change due to lactose fermentation will also be evident.

Procedure:

1. Perform a presumptive test on your water sample by inoculating approximately 10 ml of it into the test media provided. If you are using double strength phenol red lactose broth, you should add water until you have just doubled the volume of liquid in the tube.

2. Incubate all tubes at 37^{o} C until the next lab period.

3. Observe and record your results. Phenol Red Lactose Broth has the advantage of indicating fermentation as a change in color from red to yellow, in addition to gas production. Other media change color in response to acid production and may show fluorescence when exposed to ultra violet light if *Escherichia coli* is present.

Confirmatory Test

In addition to coliforms, there are other bacteria that can cause the production of acid and gas in broth containing lactose. The confirmatory test is a means of confirming that the gas produced is actually due to coliforms. The broth cultures that are positive are streaked on EMB agar and the plates examined at 24 hours for colonies typical of *Escherichia* or *Enterobacter* or both (*Escherichia, Klebsiella* and *Citrobacter* colonies are dark with green iridescence, while *Enterobacter* colonies have dark centers with light peripheries). The presence of either or both colony types constitutes a positive test. In practice, any growth on the EMB agar calls for completion of the final test.

Procedure:

1. Streak a plate of EMB agar with bacteria from your positive presumptive test tube. If your tube was not positive, streak from the tube of another student.

2. Incubate your plates at 37^{o} C for 24 hours and then examine them for the presence of colonies. Colonies of *Escherichia* are usually flat with a metallic green sheen when growing on EMB. *Enterobacter* colonies also develop a green sheen, but tend to do so only at the center. They typically have pale or light colored borders, and so are said to resemble "fish-eyes". **Note** - *Escherichia coli* are not the only type of organisms that will produce dark, flat colonies with a metallic green sheen on EMB agar plates (as indicated above).

Completed Test

Proof that the colonies on the EMB agar plate are coliform is obtained by completing the test. The suspected colonies are inoculated once again into a lactose broth, and are also slanted onto nutrient agar. Both are incubated at 37^{o} C for 24-48 hours. If the broth shows gas, and the colonies on the slant are composed of Gram-negative rods without endospores, it is concluded that coliforms were present.

Name_____ **Lab Section**_____

WORKSHEET
Exercise 25
Bacteriological Examination of Water

Goals: _____
_____

Materials & Methods:

1. Presumptive Test
   Date of sample collection: _____ Source of water: _____
   Medium used: _____
   Incubation temperature: _____ Duration of incubation: _____

2. Confirmatory Test
   Date of sample collection: _____ Source of water: _____
   Medium used: _____
   Incubation temperature: _____ Duration of incubation: _____

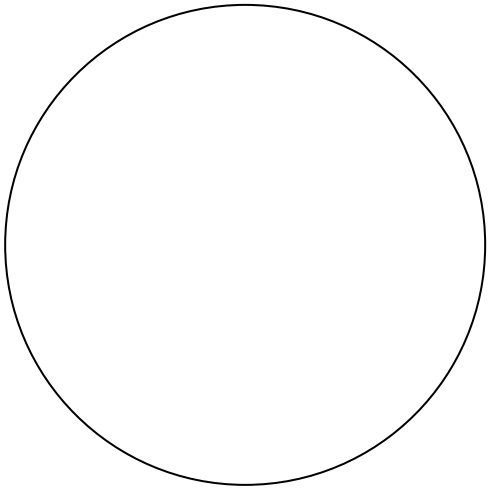
3. Completed Test
   Date of isolation (date "streaked"): _____
   Medium used: _____
   Incubation temperature: _____ Duration of incubation: _____
   Date of Gram stain: _____

Data & Results:

1. Presumptive Test
   Color of medium: _____ Gas present? _____
   Was this test positive or negative for coliforms? _____

2. Confirmatory Test
   Colony morphology: _____
   _____
   _____
   Was this test positive or negative for coliforms? _____

3. Completed test
   Color of broth medium _____ Gas present? _____
   Colony morphology on agar? _____
   _____
   Gram stain results _____
   Was this test positive or negative for coliforms? _____



Gram Stain Observations			
Total Magnification:			
Length:	lines x	μm/line =	μm
Width:	lines x	μm/line =	μm
Notes:			

Conclusions:

_____
_____
_____
_____
_____
_____

Questions:

1. Why are the tests used in the bacteriological examination of water designed to test for *E. coli*, a relatively non-pathogenic type of bacteria, rather than some of the more serious pathogens?

2. What three tests are included in the "Standard Methods" for the bacteriological examination of water.

3. Why is lactose containing broth and agar use to detect bacterial fecal contaminants in water?