WATER MICROBIOLOGY

INTRODUCTION

Mankind and water borne diseases have been on intimate terms throughout evolutionary history. However, serious water borne disease (WBD) problems really didn't begin until cities were established. A fixed population of humans confined in a compact area quickly results in a high local level of human fecal pollution. This pollution rapidly finds its way into the nearest water supply which is usually the same one the humans use for drinking and food preparation. Although man has recognized for over 5,000 years that grossly contaminated water is undesirable, only in the last 150 years have we understood the relationship between polluted water and the transmission of human disease. A number of pathogenic microbes have evolved to take advantage of water as a vehicle of distribution between hosts. These include cholera, typhoid fever, amebiasis, hepatitis A, salmonellosis, infantile & traveler's diarrhea, campylobacteriosis, a number of viruses, and H. phylori. Altogether, these diseases take a terrible toll of life every year; approximately 15,000,000 children die every year from WBD. Even today, most of the world's people lack a safe, clean water supply.

Shortly after the relationship of polluted water and WBD was recognized people began demanding fecal-free water. However, the problem was how do you measure "fecal pollution"? The "nose test" only detects pollution in gross amounts and the taste test isn't recommended. Studies on the bacterial content of the human feces soon showed that several bacteria were common to man and many other animals whose feces rountinely find their way into the water supply. It was reasoned that if one found: (a) a bacterium that resided, in nature, mainly in the gut of humans that; (b) this organism could be used as an indicator of likely fecal pollution. One such organism that fit these qualifications was a G- bacterium that was eventually named Escherichia coli. E. coli has a number of characteristics that make it a suitable fecal indicator.

* Not only is it found in human gut, but it is also found in the gut of a whole host of other animals that can carry human-WBDs and whose feces frequently find their way into the water.
* It is able to live outside the body for a long time. Therefore, its presence in water, food etc., indicates likely fecal pollution over aprolonged period.
* It has the rather unique ability to ferment the RARE sugar LACTOSE to produce acid and gas products. That is to say, very few other microbes are able to do this. So if you find a LACTOSE-FERMENTING bacteria in something the chances are high that it is E. coli and that the material is contaminated with feces.

Because of these characteristics E. coli was chosen as the indicator of fecal pollution of water. Other bacteria have been found that also serve this purpose. European nations use a gut-Streptococci, rather than E. coli as a fecal indicator. Soon diagnostic/selective media were developed that enrich for E. coli and make its detection easier and faster. Because of its role as a fecal indicator, every microbiology lab has the media and expertise to grow E. coli. Therefore, E. coli has become the most thoroughly studied bacterium on the planet and in 1998 its entire genome was sequenced.

The following procedures should help you determine what bacteria lives in various water sources. By using petri dishes that contain a growth medium one can grow bacteria. The media used to grow bacteria can be very broad, to grow a large number of bacteria varities, or very selective, to grow specific types of bacterai. In this experimnet you will use two types a media a broad medai and a selevtive media.

PROCEDURE:

Work in table groups for this lab. Determine which sample each table will be analyzing. If conditions do not permit soil samples have already been collected for you. There will be samples obtained for you as well as bottles if additional sampeling is needed.

Part 1 Dilutions

Dilutions are necessary in order to lower the concentrations of bacteria that may live in your sample. If there are to many bacteria in your sample you may unable to count or identify the bacteria due to the large numbers. So dilutions are performed to "thin" your sample so it is eaiser to analyse your sample

1) Obtain 2 dilution bottles which contain 99mLs of sterile distilled water.

2) Label one bottle A and the other B

3) Rember to be aseptic or very clean in the handeling of these samples. By practicing clean or aseptic methods we also insure that we do not add any additional to our sample and we do not come incontact with our sample

4) Be sure to shake your water samples prior to diluting. This will insure a uniform mix of your sample..

5) Using a sterile pipett take 1ml from your water sample jar and place it in the dilution botle you marked A. This will be a 1 to 100 dilution.

6) Using a sterile pipett take 1ml from your dillution bottle A and place it in the dilution botle you marked B. This will a 1 to 10,000 dilution.

Part 2 General Bacteria Growth

To see what diffrent types of bacteria are contained in your sample you will be using a media called nutrient agar. Nutriant agar media is used since many diffrent types of bacteria will grow on this media.

1) Obtrain 3 nutrient agar plates and 3 sterile pipetts.

2) Label one petri dish *orginal sample*, one *1 to 100 dilution* and the last dish *1 to 10,000 dilution* Also write your table number on the plate.

3) Using aseptic technique remove a half fo a milliter or 0.5mL of your orginal sample with the sterile pipett. Remove the lid of your plate and dispence it on your media.

4) Repeat 3 with your 1 to 100 and 1 to 10000 dillutions.

5) Place your plates in a 37C incubator. You will incubate the sample for 24 to 48 hours. If your class does not meet in this time your samples will be placed in a refrigerator to preserve them untill your next class meeting.

6) On the question sheet draw a diagram of the growth on your plates.

Part 3 Sprcific Bacteria Growth

To see if a specific bacteria is contained in your sample you will be using a media called EMB selective, this media is specific for colifrom bacteria. EMB agar will grow diffrent types of bactera, if the EMB media changes color under a plaque or growth of bacteria, that bacteria is coliform

1) Obtrain 3 EMB plates and 3 sterile pipetts.

2) Label one petri dish *orginal sample*, one *1 to 100 dilution* and the last dish *1 to 10,000 dilution* Also write your table number on the plate.

3) Using aseptic technique remove a half fo a milliter or 0.5mL of your orginal sample with the sterile pipett. Remove the lid of your plate and dispence it on your media.

4) Repeat 3 with your 1 to 100 and 1 to 10000 dillutions.

5) Place your plates in a 37C incubator. You will incubate the sample for 24 to 48 hours. If your class does not meet in this time your samples will be placed in a refrigerator to preserve them untill your next class meeting.

6) On the question sheet draw a diagram of the growth on your plates.

Laboratory Questions

Student_____ **Section**_____

Date_____

1) Explain why E. coli was chosen as the indicator of fecal pollution?

2) What circumstances in human life style made water pollution so serious?

3) Why did the EMB media changed color?

4) What is the improtance of testing beaches and pools for colirorm

5) If you are in the wilderness and do not have a water purifer why is in important to boil your water prior to drinking it?