Factors Effecting the Growth of Micro-Organisms

Introduction

The growth of micro-organisms, like that of human beings, is affected by the environment in which they are subsiding. This can be, and is on a regular basis, used to the advantage of the microbiologist and/or the clinician.

Micro-organisms require growth factors such as amino acids, carbohydrates, lipids and nucleic acids for growth. They also require minerals, salts, buffers and vitamins. If anaerobic bacteria are to be grown in aerobic surroundings, these bacteria will require media which provide a reducing environment for their growth: thioglycollate broth, a couple of dried peas tossed into the medium, an iron nail, cooked meat medium to name a few. If the bacteria are fragile, they may require chocolate agar (nutrient agar with 10% defibrinated blood that has been heated for complete hemolysis to release the nutrients from the blood cells) and 10% CO_2 for proper growth.

Bacterial growth may be inhibited just as easily as it is stimulated. Gram positive bacteria do not like to grow in the presence of dyes such as methylene blue, eosin, crystal violet or malachite green, to name a few. Antiseptics (compounds used on living tissue to kill microbes) and disinfectants (compounds used on fomites to kill microbes) kill bacteria. Lysol, Wavicide, antibacterial Dial are three examples of disinfectants (the former two) and antiseptics (the latter one). Bacterial growth is also inhibitable by antibiotics: chemicals that disrupt the bacteria and stop their growth or kill the bacteria directly. Penicillin, a cell wall synthesis inhibitor, chloramphenicol, a translation inhibitor, and polymyxin B, a cell membrane disruptor, are a few examples of antibiotics used for this purpose.

Bacteria are osmotically active. Therefore, it seems reasonable to conclude that they grow, or not, differently on media with different concentrations of salts or other osmotic media. That is, indeed, the case as you will see at the end of this experiment.

Bacterial growth, as you recall, is under genetic regulation. By exposing bacteria to ultra-violet light (UV-light), the DNA undergoes physical and chemical changes. The ultra-violet light interacts with thymine in the DNA causing the formation of "thymine dimers". Thymine dimers are two thymine molecules from the same DNA chain which are "tweaked" by the UV-light and forced to bond with

each other -- as well as remain in the DNA chain. This causes the DNA to "bubble" up. This "bubble" acts as a "blip" in the road: just like when humans drive too fast over a speed bump, when enzymes necessary for DNA replication or for transcription read the DNA with the "bubble", the enzyme either stops or "jumps" the "bubble" causing the formation of **mutant** DNA or RNA. This mutagen is not able to carry on life processes and dies. The property of the UV-light to make these changes is called its **mutagenic** property.

Materials and Methods

Materials

Candle jar with tea candle	Nutrient agar plate	Wire loops
Bunsen burner and striker	Triplate of varying NaCl concentrations	Triplate of varying sucrose concentrations
Quadraplate of nutrient agar	Antiseptics and disinfectants	Paper disks
Incubator	EMB Agar Plate	Cotton throat swab
UV-lamp	Note card	Bacteria
Blood agar plates (2)	2 Mueller-Hinton agar plates	Antibiotic disc dispenser

Methods

Effects of Varying Solute Concentration on Bacterial Growth

Obtain one salt and one sucrose plate. Each plate is labeled on the bottom of the dish as to the concentration in each compartment. Streak a bacterium (per your professor) on both plates, e.g., use the same bacterium over all three salt agars and over all three sucrose agars. Incubate and examine over the next several lab sessions. Draw your observations in the space below:

Growth pattern on salt	Growth pattern on sucrose

Effect of Antiseptics and Disinfectants on Bacterial Growth

Obtain a quadraplate of nutrient agar, 4 paper discs, four disinfectants/ antiseptics and a bacterium. Streak the bacteria over all 4 of the compartments. Pick up the paper discs individually and "soak" them with the antiseptic/disinfectant for your study one at a time. After each disc is soaked, place it in the center of the compartment. Tap it gently with your forceps to make certain it sticks to the media. REMEMBER: label each quadrant so you know which reagent is where next week. Draw your observations in the space below:

Growth pattern in presence of antiseptics/disinfectants

Effects of UV-Light on Bacterial Growth

Obtain one nutrient agar plate and a bacterium (per your professor). Follow the instructions your instructor gives you about streaking the bacteria, or not, on the agar. Follow the instructions that your instructor gives you about how long to place it under the UV-light.

In general, the streaked agar plate is to be placed beneath the UV-light WITH THE LID OFF the Petri dish. Place a note card over one half of the plate and leave the plate beneath the lamp as instructed. Next week, compare ALL of the plates and draw your observations in the space provided below:

Growth pattern following exposure to UV-light

Effect of 10% CO_2 on Bacterial Growth

Obtain two blood agar plates, a cotton throat swab and the candle jar with tea candle. Have your partner swab your throat. After your partner has swabbed your throat, streak your plates with your throat sample over half of each plate – your partner will do the same on the unstreaked half remaining. Place one of the plates in the candle jar and the other in the incubator – label appropriately. When all the plates are in the candle jar, light the tea candle and place it on top of the media. Close and tighten the lid to make a seal. As soon as the candle has gone out, place the whole apparatus in the incubator WITHOUT removing the lid. Examine both plates next week in lab. Draw your observations below:

Growth pattern: Aerobic	Growth pattern: Anaerobic

What do you notice about the aroma from each sample?

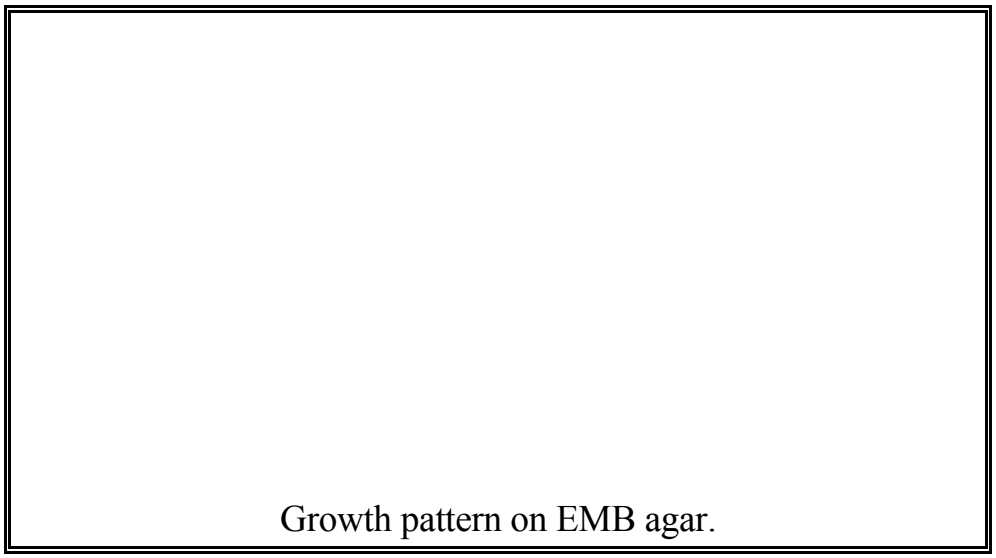
Effects of Antibiotics on Bacterial Growth

Obtain two Mueller-Hinton agar plates, your throat swab and a bacterium (per your professor). Streak the bacteria as directed by your professor. Place the antibiotic disc dispenser over streak and depress once; do the same with the dispenser over your other streak. Replace the dispensers. Tap each disc lightly with the end of your loop to make certain they stick to the media. Replace the lids, invert the plates and place in the incubator for examination next week. Draw your observations (measure the zones of inhibition in cm, too) in the spaces below:

Plate 1 – Antibiotic Sensitivity	*Plate 2 – Antibiotic Sensitivity*

Effect of Dye Presence on Bacterial Growth

Obtain one EMB agar bi-plate. On one half, gently press your thumb BEFORE you've hand-washed. On the other half, gently place your thumb AFTER you've handwashed. Place the plate in the incubator. Draw your observations in the space below at the next lab period:



Growth pattern on EMB agar.

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