**Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

**PHYSCIAL AGENTS OF MICROBIAL CONTROL**

**Preparation for Physical Agents of Microbial Control Lab Exercise**

Label 4 tubes of TS broth with the following: ER, BR, EF, BF.  Inoculate the "E" tubes with *E. coli*, and the "B" tubes with *B. subtilis.* Tubes ER and BR will be placed in the refrigerator until the next lab period.  Tubes EF and BF will be placed in the freezer until the next lab period.

Label 2 empty petri plates ED and BD.  With a sterile cotton swab, smear the "E" plate with *E. coli* broth, and the "B" plate with *B. subtilis* broth*.* Tape the plates closed and place them in the designated drawer until the next lab period.

**Materials per team (2 or 4 people):**

* 8 sterile tryptic soy broth tubes
* 2 empty sterile petri dishes
* melted TSA media
* 6 regular TSA plates
* 5 TSA plates with varying salt concentrations (0%, 3%, 6%, 9% 12%)
* 5 sterile cotton swabs
* *E. coli, B. subtilis* cultures

**Procedure:**

**Part I: Temperature**  
    Label 8 of your tubes of TS broth with name, date, and code as follows  
    (E= *E. coli* andB= *B. subtilis)*:

* EC and BC to be inoculated and incubated for 48 hours, then observed as control tubes.
* ERT and BRT to be inoculated and kept at room temperature for 48 hours, then observed.
* E56 and B56 to be inoculated and set in a 56o C water bath for 10 minutes, then incubatedfor 48 hours and observed.
* EB and BB to be inoculated and set in a boiling water bath for 10 minutes then incubated 48 hours and observed.

Move to the incubator the 4 tubes inoculated in the previous lab:

* EF and BF (frozen in previous lab) to be incubated for 48 hours and observed.
* ER and BR (refrigerated in previous lab) to be incubated for 48 hours and observed.

**Results:**

|  |  |  |
| --- | --- | --- |
|  | **Observations of Growth** | |
| **Temperature** | ***E. coli*** | ***B. subtilis*** |
| Freezing |  |  |
| Refrigeration |  |  |
| Room Temperature |  |  |
| Control (37 C) |  |  |
| Water Bath (56 C for 10 min.) |  |  |
| Boiling for 10 min. |  |  |

**Answer the following questions:**

What temperatures were most/least effective?

What similarities/differences exist in the two organisms growth patterns after exposure? Explain.

**Part II: Osmotic concentration**

Label the salt (NaCl) TSA plates with name, date, and meat (as assigned by the instructor).  Each group/pair should have one plate each of the following concentrations: 0%, 5%, 10%, 15% and 20%.  Using a sterile cotton swab, swab the meat (or assigned organism) and then lawn the 0% TSA plate.  Repeat for the other four concentrations, using a new cotton swab for each plate.  Incubate for 48 hours. Observe growth and make comparisons.

**Results:**

**Type of Meat/Organism: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **0%** | **3%** | **6%** | **9%** | **12%** |
|  |  |  |  |  |

**Answer the following questions:**

How is bacterial growth affected by hypertonic conditions? Explain.

What differences exist among the other groups in the class?

**Part III: Drying**

Label 2 empty petri dishes EA and BA.  With a sterile cotton swab, smear the "E" plate with *E. coli* broth and the "B" plate with *B. subtilis* broth.  Retrieve the "D" plates inoculated in the previous lab from the drawer. Overlay the "A" and "D" plates with melted agar and incubate for 48 hours.

**Results:**

|  |  |  |
| --- | --- | --- |
| **Observations** | **Air Dry** | **48 hour** |
| ***E. coli*** |  |  |
| ***B. subtilis*** |  |  |

**Answer the following questions:**

What effect, if any, did the drying out of the bacteria have on its growth?

What similarities/differences exist among the two organisms and their ability to withstand desiccation?

**Part IV: Effect of ultraviolet radiation**

Two lab tables should label a set of agar plates each:

        Bacillus closed, UV   (10 min.)  
        Bacillus opened, UV   
        Bacillus, cut-out, UV  
  
        E. Coli, closed, UV  
        E. coli, opened, UV  
        E. coli, cut-out, UV

Two other lab tables should label a set of agar plates each:

        Bacillus closed, FL   (10 min.)  
        Bacillus opened, FL   
        Bacillus, cut-out, FL  
  
        E. coli, closed, FL  
        E. coli, opened, FL  
        E. coli, cut-out, FL

  Lawn the plates with either E. coli, or B. sub. as labeled.

The UV labeled plates will be placed under the sterile guard hood and exposed to the UV light for 10 minutes.  Those labeled "closed" will be exposed with the lids on, and those labeled "open" will be exposed with the lids off.  Those labeled "cut out" will be covered by a cardboard cut out in any shape design.  (these lids will also be removed at the start of exposure)

The FL labeled plates will be placed in the Sterile guard hood and exposed to the fluorescent work light for 10 minutes.

All plates will be incubated for 48 hours, observed and then comparisons should be made.

**Results:**

**Ultraviolet (UV) Light Exposure**

|  |  |  |
| --- | --- | --- |
| **Condition** | ***B. subtilis*** | ***E. coli*** |
| Open |  |  |
| Closed |  |  |
| Cut-out |  |  |

**Fluorescent Light (FL) Exposure**

|  |  |  |
| --- | --- | --- |
| **Condition** | ***B. subtilis*** | ***E. coli*** |
| Open |  |  |
| Closed |  |  |
| Cut-out |  |  |

**Answer the following questions:**

What effect does UV/FL light have on the growth of bacteria?

What differences exist among the types of organisms observed? Explian.

What are the limitations when considering UV/FL use on the viability of microorganisms?