**02/05/2015 identifying bacteria and observing antibiotic resistance**

**Purpose**

The purpose of this lab experiment was to observe prokaryotic bacteria and the growth of bacteria on the agar plates and also to understand the effects of antibiotic (tetracycline) resistance.

**Materials and Methods**

For this lab experiment, we used agar plate samples that were diluted the previous week, water, microscope oil, gram iodine, 95% alcohol, safranin stain, crystal violet, Kim wipe, slide-cover slip. For this experiment, two procedures were used namely wet mount and gram stain procedure. For the wet mount experiment, we sterilized a lope over a flame and used it to scrape a portion of bacteria from agar plate. Mixed it with drop of water on the slide. Placed a cover slip and observed it under the microscope using the 10x and then the 40x objective. For the gram stain experiment, a loop was sterilized over flame and used to scrape a portion of the bacteria from agar plate onto slide. A drop of water was added to sample. Slide was slowly passed over flame still water dried. Covered bacteria with crystal violet, let it stay for a minute and rinse stain with distilled water. Covered bacteria smear with gram’s iodine for a minute and rinsed with distilled water. Covered bacteria smear with 95% alcohol for 10-20 sec. covered smear with safranin for 20-30 sec and rinsed using distilled water. Used Kim wipe to clean excess water and allowed to dry. Places cover slip on slide and viewed under microscope using low magnification and then used 40x and the 100x oil immersion objectives.

**Data and observations**

Do you think any archaea species will have grown on the agar plates? Why or why not? I do not think any archaea species will have grown on the agar plate because the environment is not suitable for living. Archaea prefer to live in extreme environments such as hot springs or at the bottom of the ocean. Looking at my Hay Infusion Culture, I observed that there is less water in the jar after a week and is smells less than it did the previous week.

**Table 1: 100-fold Serial Dilution Results**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Dilution** | **Agar type** | **Colonies counted** | **Conversion factor** | **Colonies/ml** |
| 10-2 | Nutrient | Lawn | × 103 | lawn |
| 10-4 | Nutrient | 60 | × 105 | 60 × 105 |
| 10-6 | Nutrient | 2 | × 107 | 2 × 107 |
| 10-8 | Nutrient | 0 | × 109 | 0 × 109 |
| 10-2 | Nutrient + tet | Lawn | × 103 | Lawn |
| 10-4 | Nutrient + tet | 45 | × 105 | 45 × 105 |
| 10-6 | Nutrient + tet | 1 | × 107 | 1 × 107 |
| 10-8 | Nutrient + tet | 0 | × 109 | 0 × 109 |

There were more bacteria/colony on the plates without antibiotics than they were on the plates with antibiotics. This indicates that tetracycline prevented the growth of bacteria. I observed that two species of bacteria are unaffected by tetracycline. According to [Tetracycline Antibiotics](http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CB4QFjAA&url=http%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fpmc%2Farticles%2FPMC99026%2F&ei=0qjTVMDmI8jbsATetYKQAQ&usg=AFQjCNHPyaSsM8CbkSpp16R14ZAoTCCUbw)  article, tetracycline kills inhibits protein synthesis by preventing the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site. If bacteria are unable to undergo protein synthesis, this could account for why we only had gram negative bacteria and not gram positive

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Letter | Colony label | Plate type | Colony description | Cell description | Gram + or Gram - | Additional notes |
| A | 10-2 | Tetracycline | Smooth, glistening, rhizoid colony. Flat/convex | n/a | Gram - | 0.1-0.4cm |
| B | 10-4 | No tetracycline | Irregular, raised, smooth, shiny | n/a | Gram - | 0.3cm |
| C | 10-4 | Tetracycline | Circular. Smooth, shiny colony | n/a | Gram - | 0.5cm |
| D | 10-2 | No tetracycline | Irregular, smooth, raised shiny | n/a | Gram - | 8.5cm |

The above table shows the characteristics of bacteria from agar plate observed under a microscope. We were unable to do cell description due to time management and difficulties viewing bacteria under the microscope.

**Conclusion**

One of the challenges that I faced in this particular lab was viewing the organisms under the microscope. It was extremely difficult to actually see the bacteria which made filling the chart difficult. In the future, I propose to manage time wisely and for students to use prepared slides because it was difficult to view anything using the wet mounts slides, and to also try to understand the concepts of the lab and not just do it for the sake of doing a lab because when you know what you are doing, the experiment is easier to conduct.

YTM