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|  | **Materials Needed:**   |  |  |  |  | | --- | --- | --- | --- | | 250mL beaker (4) | Cheesecloth | Hole-punch | Scale | | 2000mL beaker (3) | Chromatography paper | Hot plate | Sink | | Aluminum Foil | Cotton swabs | Living Bacillus  cereus | Tab water | | Aprons | Distilled water | Matches or striker | Test tubes | | Autoclave | Erythromycin 400mg tablets | Metric ruler | Thermometer | | Bio-hazardous bag | Garlic (1 bulb) | Micro pipette | Toothpicks | | Bleach | Glass stirring rod | Micro pipette tips | Trash can | | Bunsen burner | Gloves (surgical/rubber) | Micro-scale spot plate (2) | Tweezers | | Laboratory Sterilizer | Graduated Cylinder | Mortar & pestle | Wax pencil | | Calculator | Growth medium (tryptic-soy agar) | Petri dishes (4) |  |   **Procedures:**  1- Put on apron and surgical gloves. Sterilize all glassware with the C2250 Sybron/Barnstead Laboratory Sterilizer:   * Open door. * Depress "Fill" button until water is one inch from the front of chamber. * Load glassware into sterilizer. * Set temperature to 270 ( F * Set vent control to " * Close and lock door. * Turn cycle timer to 10 and then turn to desired time (3 minutes). * When cycle is complete, open door slightly. (Prevent burns by opening door slightly to allow residual steam to dissipate.) * Turn cycle timer to 10 then to desired drying time. * When cycle is complete, carefully open door. Unload glassware cautiously.   2- Disinfect lab area with a 25% bleach solution mixed with tab water in a 2000mL beaker. Be sure to wear rubber gloves to protect hands from bleach.  3- Label two pairs petri dishes as follows:  4- Prepare agar plate (details comes with bacteria)   * Open packet and empty the powder into a 2000mL glass beaker. * Measure out one liter of distilled water into a second 2000mL glass beaker. Add much of the water into the first glass beaker over the powder. With the remaining water, rinse out the packet and add to the first glass beaker. * Place first beaker on hot plate and stir while heating. Heat until powder completely dissolves. * Allow 10 to 15 minutes to cool. * Pour enough mixture into the petri dishes to cover the bottom of the dishes. * Let cool until the agar solidifies. * Discard the excess mixture.   **While the agar is cooling:**  (5) Label wells of a plastic micro-scale spot plate A, B, C, D, E, and F. (Figure 8)  (6) Crush three cloves of garlic with mortar and pestle completely. (Figure 9)  (7) Transfer the crushed garlic onto the cheesecloth, and squeeze the garlic juice onto tin foil.  (8) Using the micro pipette, withdraw 20(L of garlic juice and place it in the well labeled A.  (9) Transfer 15(L of garlic juice into well B, 10(L into well C, and 5(L into well D. (Figure 10)  (10) With a new pipette tip, add 5(L of distilled water into well B, 10(L into well C, 15(L into well D, and 20(L into well E. Stir mixtures B, C, and D with different toothpicks.  (11) Punch out six discs of chromatography paper with the hole-punch.  (12) Place a disc in each of the wells and let soak for 15 to 20 minutes.  **While chromatography discs are soaking:**  (13) Be sure to have read the details and safety instructions that came with the bacteria.  (14) Put a sterile cotton swab into the test tube containing Bacillus cereus to obtain the bacteria. (Figure 11)  (15) Establish a bacterial lawn with the streak-plate method:  A. Lift lid carefully to a 45( angle and lightly streak the cotton swab back and forth evenly across the entire surface of the petri dishes. Be sure to swab the bacteria to the edges of the dish. (Diagram 1; Figure 12)   |  |  | | --- | --- | |  |  |   B. Rotate the petri dishes 45deg and swab at right angles to the first swab. (Diagram 2; Figure 12)  Diagram 1 Diagram 2   |  |  | | --- | --- | |  |  |   (16) Using tweezers, obtain a disc from well A and place it on the region of the petri dish labeled A. Be sure that the disc is in full contact with the agar gel.  (17) Disinfect the tweezers with Bunsen burner. Allow the tips of the tweezers to turn red at least two times. Then, let cool before further use.  (18) Repeat Steps 16 and 17 for wells B, C, D, and E. (Figure 13)  (19) Invert the petri dishes and incubate in autoclave overnight at approximately 37C.  (20) Crush a 400mg tablet of erythromycin into fine dust with mortar and pestle.Measure 100mL of distilled water into each of four 250mL beakers.  (21) Mass 0.05g of erythromycin with scale and add powder to one of the four 250mL beakers.  (22) Stir the mixture and try to dissolve the powder.  (23) Repeat steps 20 & 21 for 0.10g, 0.15g, and 0.20g of erythromycin.  (24) Using a different micro pipette tips for each, extract 20(L of each mixture and place into wells A,B,C, and D.  (25) Using a different pipette tip to add 20(L of distilled water into well E.  (26) Soak a chromatography disk in each of the wells for 10 to 15 minutes.  (27) Using tweezers, obtain a disc from each well and place onto the appropriate regions of the the petri dishes.  (28) Place a dry chromatography disk onto region F.  (29) Invert the petri dishes and incubate in autoclave overnight.  (30) Disinfect lab area again using the bleach solution. Discard it after use in the sink with plenty of water. (Figure 14)  The next day:   1. Put on apron and surgical gloves. 2. Disinfect lab area using 25% bleach solution. 3. Open autoclave and retrieve petri dishes. 4. Observe and measure the diameter of the zone of inhibition for each group. 5. Record data in Table Raw Data. 6. Repeat Steps 1-5 for several days. 7. Rpeat experiment 10 times.   **To discard bacteria and petri dishes**:   1. Increase the temperature of the autoclave to 100(C. Place the petri dishes in autoclave overnight. 2. The next day, pour 100% bleach into petri dishes and wait for the gel to disintegrate. 3. To the original test tube of Bacillus cereus, add 100% bleach until the tube is full. Close the cap tightly. 4. Discard petri dishes and test tube of Bacillus cereus in a bio-hazardous bag.   In this experiment, there were eight experiemental groups - Groups A, B,C, and D tested with garlic, and Groups A, B, C, and D tested with erythromycin. Each was varied by the concentration of garlic or erythromycin applied. In both instances with garlic and erythromycin, there were also Groups E and F added. Group F consisted of dry chromatography disks, as it is to act as a control group for the experiment. Group E, consisting of chromatography disks soaked in distilled water, was included to account for any effects distilled water may have on the cultures. Each group was exposed to similar test conditions - gel medium, incubation time and temperature, and size of cultures, so that the experiment was limited to only one variable. |

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