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|  | |  | | --- | | **Procedure:**  Procedures:  1.Put on apron and surgical gloves. Sterilize glassware with the C2250 Sybron/Barnstead Laboratory Sterilizer:  Open door  Depress ìFillî button until water is one inch from the front of chamber  Load glassware into sterilizer  Set temperature to 270(F  Set vent control toî  Close and lock door  Turn cycle timer to 10 and then turn to desired time (approx.3 minutes)  When the cycle is complete the indicated light should turn off  Open the door slightly to allow steam to slowly escape, preventing burns  Unload glassware  2. Disinfect lab area with a 25% beach solution mixed with tap water in one of the 2000mL beakers. Use a sponge and rubber gloves.  3. Label 30 petri dishes and divide each dish into equal sections of 3. Label two petri-dishes A, B, C, D, E, F.  Label the remaining 30 petri-dishes the same way  4.Prepare agar  prepare the agar according to directions stated on the container.  Usually, it is preferable to double the recipe to not run out of agar  While the agar is cooling  5. Label wells of a plastic micro-scale spot plate A, B, C, D, E, and F  6. Slice the about 3g of ginger into approximately 5cm disks.  7. Place the sliced garlic into the mortar and pestle and begin crushing the disks until extract is noticeable. Then place the ginger and extract onto the cheesecloth.  8.Transfer the ginger extract onto aluminum foil by squeezing the cheesecloth.  9.Using the micro pipette, withdraw 20L of ginger juice and place it in the well labeled A  10. Transfer 15L of ginger juice into well B, 10L into well C and 5 into well D  11. Using a new pipette tip, add 5L of distilled water into well B, 10L into well C, 15L into well D and 20 L into well E. Stir mixtures B, C,D with different tooth picks.  12. Punch out 90 disks of chromatography paper with the hole punch.  13. Place 15 disks in each well and let soak for 15 to 20 minutes  While the chromatography discs are soaking:  13. Read the safety directions of the bacteria  14. Put a sterile cotton swab into the test tube containing Bacillus Cereus to obtain the bacteria.  15. Establish a bacterial lawn with the streak plate method on all of the disks.  A.Lift lid carefully to a 45 degree angle and slightly streak the cotton swab back and forth evenly across the entire surface of the petri dishes.  B.Rotate the petri dish 45 degrees and repeat part A.  16. Using sterile 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 nutrient agar gel.  17. Disinfect the tweezers with the Bunsen Burner and let cool.  18. Repeat Steps 16 and 17 for wells B, C, D, and E on all the petri dishes.  19. Invert the petri dishes and incubate in the autoclave overnight at approximately 37C.  20. Crush one 400mg tablet of erythromycin into fine dust with the mortal and pestle.  21. Add the finely crushed tablet into a 250ml beaker.  22. Add 100ml of distilled water into the 250 ml beaker.  23. Stir the mixture for approximately 5 minutes to ensure even dilution and dissolving.  24. Using different micro pipette tips for each, extract 20L the mixture and place it in well A then extract 15L of the mixture and place in well B. Then extract 10L of the mixture and place in well C. Then extract 5L of the mixture and place it in well D.  25.With a new pipette tip transfer 5L of distilled water into well B, 10L into well C, 15 into well D and 20 L into well E.  26. Punch out 90 chromatography disks and place 15 in each of the wells.  27. Let the disks soak for approximately 10 to 15 minutes  28. Place a dry chromatography disk into each area labeled F.  29. Invert these chromatography disks and place them in the autoclave overnight.  30. Disinfect lab area with bleach solution to kill remaining bacteria.  31. Rinse off the lab station with a damp sponge.  The next day:  1.Put on lab apron and surgical gloves  2.Disinfect lab area using the 25 percent bleach solution  3. Open autoclave and retrieve all 60 petri-dishes  4.Observe and measure the diameter of the zone of inhibition for each group.  To discard bacteria and petri dishes.  1. Let bacteria stand for several days until the agar completely dries up.  2. Pour 25 bleach solution into each petri dish and wait for gel to disintegrate.  3.Discard petri dishes in a bio-hazardous bag.  This experiment consisted of eight experimental groups: Groups A, B, C, D tested with ginger and Groups A, B, C, D tested with erythromycin. Each letter in each group had varied concentrations of ginger and erythromycin respectively. The concentrations were A-100%, B-75%, C-50%, D-25%. As a control groups E and F were applied where E was basically distilled water and F was just a chromatography disk alone. Each group was exposed to similar test conditions which included similar growth medium (nutrient agar), constant autoclave temperature, size of cultures, and incubation. In conclusion, the experiment was limited to one variable and all controls were exercised. | | |
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