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|  | Materials Needed:     |  |  | | --- | --- | | 2000mL beaker  250mL beaker (2)  6 well Micro-scale spot plate (3)  Aluminum Foil  Aprons  Autoclave  Bio-hazardous bag  Bleach  Bunsen burner  Calculator  Cheesecloth  Chives (4 bunches)  Chromatography paper  Distilled water  Garlic (1 bulb)  Glass stirring rod  Gloves (surgical/rubber)  Goggles  Graduated Cylinder  Growth medium (tryptic-soy agar)  Hole-punch  Hot plate | Laboratory Sterilizer  Leek (2)  Living Bacillus cereus  Matches or striker  Metric ruler  Micro pipette tips  Micro pipette  Mortar & pestle  Onion (1 bulb)  Petri dishes (6)  Scale  Shallots (2 bulbs)  Sink  Tap water  Test tubes  Thermometer  Toothpicks  Trash can  Tweezers  Wax pencil  Wire Loop |   (1) Wash hands, put on apron and gloves, and setup lab area.  (2) 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. If no water comes out, add distilled water to the funnel at the top.  · Load glassware into Sterilizer.  · Set temperature to 270 degree F.  · Set vent control to the scissors symbol.  · Close and lock door.  · Turn cycle timer to 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 3 minutes, drying will automatically begin.  · When cycle is complete, carefully open door. Unload glassware.  (3) Disinfect lab area with a 25% bleach- tab water solution in a 2000mL beaker. Be sure to wear rubber gloves to protect hands from bleach.  (4) Label and date petri dishes for identity.  (5) Prepare agar plates:  · The agar:water concentration per dish is about 0.73 gram powder : 16.6 mL water.  · Open agar powder packet and empty the right amount of powder into a 250mL glass beaker.  · Use gradual cylinder to measure out the corresponding amount of water and pour into the beaker.  · Place beaker on hot plate and stir with glass rid while heating. Heat until powder completely dissolves.  · Allow to cool until touchable (make sure hot beaker is not placed directly on a cold surface).  · Lift the lid of the dish at a 45 degree angle to prevent air contamination and pour enough mixture into the petri dishes to cover the bottom of the dishes.  Figure 1: Hold the lid at a 45 degree angle  · Close the lid tight. Let cool until the agar solidifies.  · Turn the dishes upside down to prevent condensation on the cover from dripping onto agar.  · Discard the excess mixture in trash can after it solidified.  (6) While waiting for the agar to solidify, obtain the juices from garlic, chives, leek, onion, and shallot  Figure 2: crushing onions using the mortar and pestle  Figure 3: Squeezing juice from onions using a cheesecloth  Garlic, shallots, and onions:  · remove the dry, papery skin  · cut into small chunks  · crush each separately with mortar and pestle until it's a fine pulp (be sure to clean the mortar and pestle before crushing a different plant)  · Place a large square of aluminum foil onto the counter top.  · Transfer the prepared plant onto the cheesecloth, and squeeze the juice onto aluminum foil.  Chives:  · rinse under running tap water to remove sand and grit  · cut into small slivers  · Place a large square of aluminum foil onto the counter top.  · Transfer the cut chives onto the cheesecloth, and squeeze the juice onto aluminum foil.  Leeks:  · cut off the roots and green top, keeping only the white stalk  · rinse under running tap water to remove sand and grit  · cut into small chunks  · Place a large square of aluminum foil onto the counter top.  · Transfer the cut leeks onto the cheesecloth, and squeeze the juice onto aluminum foil.  (7) Label the each row of the spot plate with the names of the 5 substances and label each well with a different letter A (100%), B(75%), and C (50%).  G=garlic O=onion C=chives S=shallots L=leeks  Figure 4: Soaking chromotography paper in varying concentrations of garlic and onion  (8) Using the micro pipette, withdraw the appropriate amounts of distilled water and juice to create a 100%, 75%, and 50% juice concentration according to the table below:     |  |  |  | | --- | --- | --- | | Well | Distilled Water (uL) | Juice (uL) | | Chive A | 0 | 200 | | Chive B | 50 | 150 | | Chive C | 100 | 100 | | Garlic A | 0 | 200 | | Garlic B | 50 | 150 | | Garlic C | 100 | 100 | | Leek A | 0 | 200 | | Leek B | 50 | 150 | | Leek C | 100 | 100 | | Onion A | 0 | 200 | | Onion B | 50 | 150 | | Onion C | 100 | 100 | | Shallot A | 0 | 200 | | Shallot B | 50 | 150 | | Shallot C | 100 | 100 | | Control | 200 | 0 |   · measure out the amount suggested in the table using the micro pipette, making sure you use a new pipette tip every time you obtain a new substance.  · stir each of the wells with a new toothpick to ensure an even concentration.  (9) Punch out 17 discs of chromatography paper with the hole-punch. Place a disc in each of the wells using tweezers. Let soak for 15 to 20 minutes. Save one dry disc for control.  (10) While chromatography discs are soaking, prepare the bacteria lawn:  · Be sure to have read the details and safety instructions that came with the bacteria.  · Set-up Bunsen burner.  · Flame the loop wire until it turns red for 2 times.  · Remove cap of the bacteria culture test tube. Flame the mouth by passing it 2 or 3 times through the burner flames. Hold the tube almost parallel to the table top to reduce the possibility of air-borne contaminants.  · Put the loop wire into the test tube to obtain bacteria (make sure the loop has cooled down).  · Close the cap of test tube.  · Lift lid to a 45 degree angle  · Swab the loop evenly onto the agar using the streak-plate method (making sure to swab the bacteria to the edges of the dish)  Figure 5: Swap the wire loop evenly onto the agar  · Rotate the petri dishes 90 degrees and swab at right angles to the first swab.  Figure 6: Rotate the plate 90 degrees and once again apply the bacteria  (11) Label each dish with the substance name and letter A, B, and C in three separate regions.  (12) Using tweezers, obtain a disc from the well and place it on the corresponding region of the petri dish (make sure that the disc is in full contact with the agar gel).  (13) 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. Repeat step 11 to 13 until a disc from each well has been used. Also put in the two control discs.  (14) Turn petri dishes upside down and incubate them in autoclave overnight at approximately 37 degrees C.  (15) Disinfect lab area again using the bleach solution. Discard bleach in the sink with plenty of water and waste materials in bio-harzard bag.  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 (include special physical appearance of the discs).  (6) Repeat experiment 10 times.  To discard bacteria and petri dishes:  (1) Pour 100% bleach into each dish. Wait until the agar completely disintegrate, and pour it down the sink.  (2) To the original test tube of Bacillus cereus, add 100% bleach until the tube is full. Close the cap tightly. Wait until the agar completely disintegrate, and pour it down the sink.  (3) Discard petri dishes and test tube of Bacillus cereus in a bio-hazardous bag.  In this experiment, there were 15 experimental groups --- Groups A, B, and C tested with each of the five substances: garlic, onion, leek, shallot, and chives. Each was varied by the concentration of the juice applied. In all instances, 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, consisted 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. |

*This Web Site is Best viewed with 256 or more colors.*

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