# **Materials**

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| --- | --- | --- | --- |
| 1000 ml beaker | Bentgrass | Gloves | Mortar & Pestle |
| 500 ml beaker (2) | Bunsen Burner | Graduated Cylinder | Paper Towel |
| Agar powder | Calculator | Hole Punch | Petri Dish |
| Ammonia | Chromatography | Hot Plate | Scale |
| Aprons | Distilled Water | Inoculation loop | Tweezers |
| Bacillus cereus | Glass Stirring Rod | Matches | Well Plate |

**Procedure**

Preparing the Agar plate

1. Open and pour 23 g of agar nutrient powder.
2. Pour 1000 ml of distilled water into a1000 ml beaker.
3. Separate the 1000 ml solution into two 500 ml beakers.
4. Plug in two hot plates and set the two 500 ml beakers on it.
5. Stir the agar solution with a stirring rod. Heat the solution until the powder/particles disappear.
6. Pour agar solution into 30 petri dishes approximately 1/3 of the petri dish high, or enough to cover the bottom of the dish.
7. Let agar solution cool off for about 15 minutes until it turns into gel.

Preparation before adding the bacteria

1. Wipe down the lab table with ammonia before starting.
2. Sterilize the tweezers and the inoculation tubes before opening the bacteria vial.
3. Wear rubber gloves

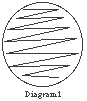
Making the Solution

(Repeat steps 15 times)

1. Obtain a well plate with six holes.
2. Take the roots of the creeping bentgrass, pacific bentgrass, and the spike bentgrass grown in 0% sulfur soil concentration.
3. Ground the roots from each of the bentgrass separately using a mortar and pestle.
4. Add 1 ml of distilled water into the mortar as the roots are being crushed to make a small solution.
5. In the well plate, put each species of bentgrass in each hole.
6. Label the well plate with (a), (b), (c), (d), (e), and (f).
7. = Pacific Bentgrass at a certain %
8. = Creeping Bentgrass at a certain %
9. = Spike Bentgrass at a certain %
10. = no solution
11. = distilled water
12. = distilled water and soil
13. Add one disk of chromatography paper to each well. (d) is just chromatography paper. Leave each circle in the solution for approximately 10 minutes.
14. Label a petri dish with the trial number and divide the dish with a wax pencil into 6 areas. Label it (a) – (f) in clockwise arrangement.

Adding the Bacteria

1. Take a vial of Bacillus cereus and sterilize the opening with ammonia.
2. While wearing gloves, take the inoculation tube and put it into the vial of bacillus cereus.
3. Stroke up and down with the inoculation tube on the agar carefully, spreading the bacteria in a criss-cross manner. Repeat process twice on each plate.
4. Do this process to each agar plate.



Adding the Chromatography paper

1. Take the well plate with 6 different chromatography paper disks and using sterilized tweezers, pick each disk up and put it on the corresponding part of the agar plate. So, (a) with (a), (b) with (b), and so on and so on.
2. Cover the plate quickly after adding the chromatography paper.
3. Let the plates alone for 24 hours after adding the chromatography disks.

Collecting the Data

1. After a 24-hour period, take the petri dishes and measure the zone of inhibition if any is seen.
2. Record in Data Chart and Graph.