

Aim of the Experiment:**ANTIBIOTIC SUSCEPTIBILITY TEST OF PROBIOTIC CULTURES****Introduction**

One method that is used to determine antibiotic susceptibility is the sensitivity disk method of Kirby Bauer. In this method, antibiotics are impregnated onto paper disks and then placed on a seeded Mueller-Hinton agar plate using a mechanical dispenser or sterile forceps. The plate is then incubated for 16 to 18 hours, and the diameter of the zone of inhibition around the disk is measured to the nearest millimeter. The inhibition zone diameter that is produced will indicate the susceptibility or resistance of a bacterium to the antibiotic. Antibiotic susceptibility patterns are called antibiograms. Antibiograms can be determined by comparing the zone diameter obtained with the known zone diameter size for susceptibility. For example, a zone of a certain size indicates susceptibility, zones of a smaller diameter or no zone at all show that the bacterium is resistant to the antibiotic. Frequently one will see colonies within the zone of inhibition when the strain is antibiotic resistant. Many factors are involved in sensitivity disk testing and must be carefully controlled. These include size of the inoculum, distribution of the inoculum, incubation period, depth of the agar, diffusion rate of the antibiotic, concentration of antibiotic in the disk, and growth rate of the bacterium. If all of these factors are carefully controlled, this type of testing is highly satisfactory for determining the degree of susceptibility of a bacterium to a certain antibiotic. The Kirby-Bauer method is not restricted to antibiotics. It may also be used to measure the sensitivity of any microorganism to a variety of antimicrobial agents such as sulfonamides and synthetic chemotherapeutics.

Materials

1. Probiotic strain
2. Mueller-Hinton agar plates
3. Antibiotic disk dispensers or assorted individual vials containing antibiotic disks
4. Sterile swabs
5. 4- to 6-hour MRS broth cultures of *Lactobacillus spp.*
6. 35°C incubator
7. Forceps
8. Metric rulers
9. Wax pencil
10. 70% ethyl alcohol and beakers
11. Bunsen burner

Procedure

1. With a wax pencil, mark the lid of each Mueller Hinton agar plate and the name of the bacterium to be inoculated.
2. The swab is immersed in the culture tube, and the excess culture is squeezed on the inner side of the test tube.
3. The swab is then taken and streaked on the surface of the Mueller-Hinton plate three times, rotating the plate 60° after each streaking. Finally, run the swab around the edge of the agar. This procedure ensures that the whole surface has been seeded. Allow the culture to dry on the plate for 5 to 10 minutes at room temperature with the top in place.
4. Dispense the antibiotics onto the plate either with the multiple dispensers or individually with the single unit dispenser.
5. Make sure that contact is made between the antibiotic disk and the culture by gently pressing the disk with alcohol-flamed forceps.
6. Do not press the disk into the agar, and do not move the disk once it is placed on the agar.
7. Incubate the plates for 16 to 18 hours at 35°C. Do not invert the plates.
8. Measure the zones of inhibition to the nearest mm for each of the antibiotics tested. Record the results in the report for exercise. For each antibiotic, determine whether the bacteria are resistant or susceptible.

Observations

Antibiotic Susceptibility Test (Susceptibility/Resistance) S/R

Name of the Isolate	Antibiotic A	Antibiotic B	Antibiotic C	Antibiotic D
Control				
Isolate A				
Isolate B				
Isolate C				

Interpretation