

Disk Diffusion Assay with Antibiotics

The day before:

- Prepare overnight cultures of the strains to test in 3mL Mueller-Hinton(MH-) Medium containing antibiotics (1:1000)
- Incubate at 37°C overnight, 220rpm agitation
- Pour MH-Agar Plates (relatively thin layer), preferentially square plates

The day of the experiment:

- Set up 10mL day cultures in MH-Medium and inoculate 1:500 with the overnight culture (no antibiotics added)
- Let grow to an OD600 between 0.4 and 0.6
- In the meantime, prepare test tubes with 10mL MH-Soft Agar (keep liquid in a 60°C water bath) and also prepare the disks:
- Choose your antibiotic concentrations not too high, so that the inhibition zones do not interfere with each other
- Put 10µl of the antibiotic concentration in a disk and let it dry before use
- When the day cultures have reached the right optical density, add 100 µl of the culture (and other additives of interest) to the liquid MH-Soft Agar, vortex and pour onto MH-Agar Plates (prepared the day before)
- Let the agar solidify
- Place the disks soaked in antibiotics (but dried) on the Plates, use disks with H₂O as a negative control
- Incubate the plates at 37°C for about 24 hours

The day after the experiment:

- After incubation, detect potential luminescence (1 and 2min exposure using Alpha Imager) and measure diameter of inhibition zones on the lawn of bacteria