Adhesion Inhibition Assay

Materials:

- LB (sterile)
- 14 ml bacterial culture tubes with caps
- Falcon tubes 15 ml & 50 ml
- multichannel pipette & pipette tips
- Agilent BioTek Epoch 2 Microplate Spectrophotometer
- 96-well Nunc plates (Thermo Fischer)
- 96-well transparent Microtiter plates (Sarstedt TC-Plate 96 Well, Standard, F (roundbottom) Cat. No. 83.3924.005)
- Yeast manna (Mannan from Saccharomyces, Sigma-Aldrich, CAS-No.: 9036888-8) in carbonate buffer (conc. 1.2 mg/mL) (sterile)
- DPBS -$CaCl_2$, -$MgCl_2$ (Gibco Cat. No. 14190-094)
- BSA: 5% Bovine Serum Albumin (Affiliate of Merck KGaA) in DPBS buffer (sterile)
- Phosphate buffer solution: PBS buffer, Tween 0.05% (Carl Roth GmbH) (sterile)
- LB agar plates
- *E. coli* ORN 178
- *E. coli* ORN 208 (negative control, *E. coli* ORN 178 *ΔfimH*)
- Eppendorf Bio Photometer
- Plastic cuvettes 1 ml
- Reservoir
- Inhibitors in DPBS

Work under sterile conditions

Methods:

Day 1 → Coat 96-well plates with yeast mannan. Add 100µL of 1.2 mg/mL yeast mannan in carbonate buffer to each of the 96 wells using a multichannel pipette. Using a sterile lid, dry the plate in the 37°C incubator for two days for the mannan to completely evaporate.

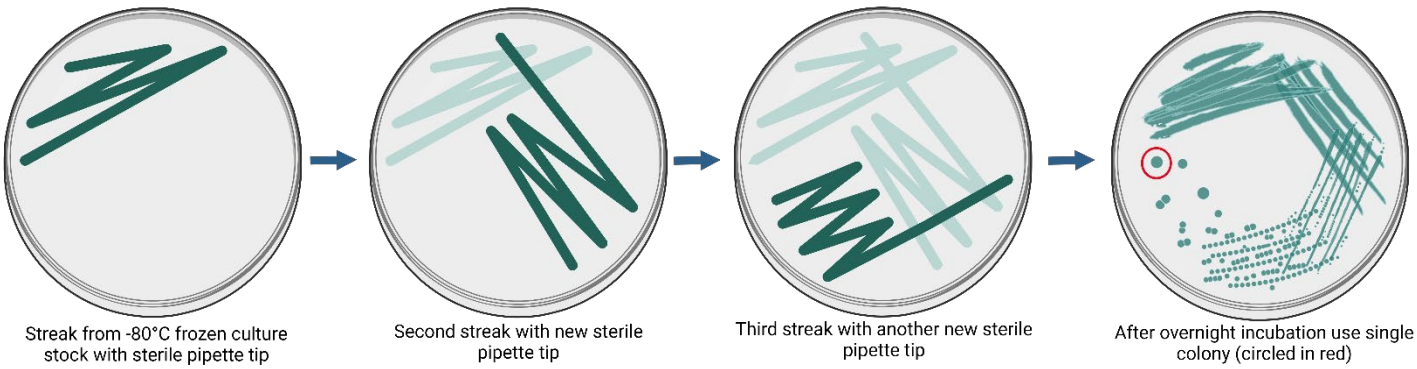

Figure 1: Single colony streak out method.

Day 2 → Streak out (single-colony method) bacterial strains (*E. coli* ORN178 and ORN 208) on LB Agar plates as shown above and allow to incubate overnight (ON) at 37°C.

Day 3 → Prepare bacterial culture by inoculating 5mL LB with single colony from plate. Prepare negative control of only LB (3mL). Grow shaking 180-250 rpm at 37°C and allow to grow to mid-exponential phase (OD_{600} 0.5) (approx. 3 hours, measuring OD every 30 min after 2 hours mark).

In the clean bench, wash the mannan coated plate first with 100µL PBST in each well, carefully suck out the liquid with the vacuum system making sure not to disturb the mannan coating. Next wash each well with 100µL DPBS, carefully suck out the liquid with the vacuum system making sure not to disturb the mannan coating. Finally add 100µL of 5% BSA in DPBS to each well. Shake the 96-well plate covered with sterile lid at room temperature at 110 rpm for 30-min to 2 hours. Suck out liquid carefully. Wash every well again with 100µL PBST, suck out liquid. Then wash every well with 100µL DPBS and carefully suck out liquid, always careful not to touch the coated wells.

Confirm that the negative control LB is still clear and sterile. Wash the liquid bacterial cultures (ORN178 and ORN208) by centrifuging for 5 min at 3000 xg. Discard the supernatant, careful not to disturb the bacterial pellet. Resuspend each bacterial pellet in 4 mL DPBS. Measure OD_{600} and adjust to an OD_{600} of 0.3-0.4.

Using a separate 96-well plate so as not to disturb the mannan coating, perform 1:2 serial dilution in DPBS of the compounds to be tested. Recommended to use mannose as a control inhibitor at same concentration as other compounds being tested. Thus fill the first well with 100µL of compound, fill the following wells where the serial dilution will take place with 50µL of DPBS. **Remember to switch tips when going down in dilution series.** Then take 50µL of the compound from the first well and mix with the second well. After thorough mixing, take 50µL from current well of mixed solution and mix in the subsequent well. Continue serial dilution until the last well in the compound series and throw away the last 50µL from the last well to ensure equal volumes. After dilution set up transfer the solution using a multichannel pipette and switching tips between samples, to the mannan coated plate. Add 50µL of 0.35 OD_{600} ORN178 to all of the transferred compounds. In wells A1-6 add 50µL of DPBS. Add 50µL of the 0.35 OD_{600} ORN178 to wells A1-3. Add 50µL of the 0.35 OD_{600} ORN208 to wells A4-6. The following layout can be used for sample arrangement.

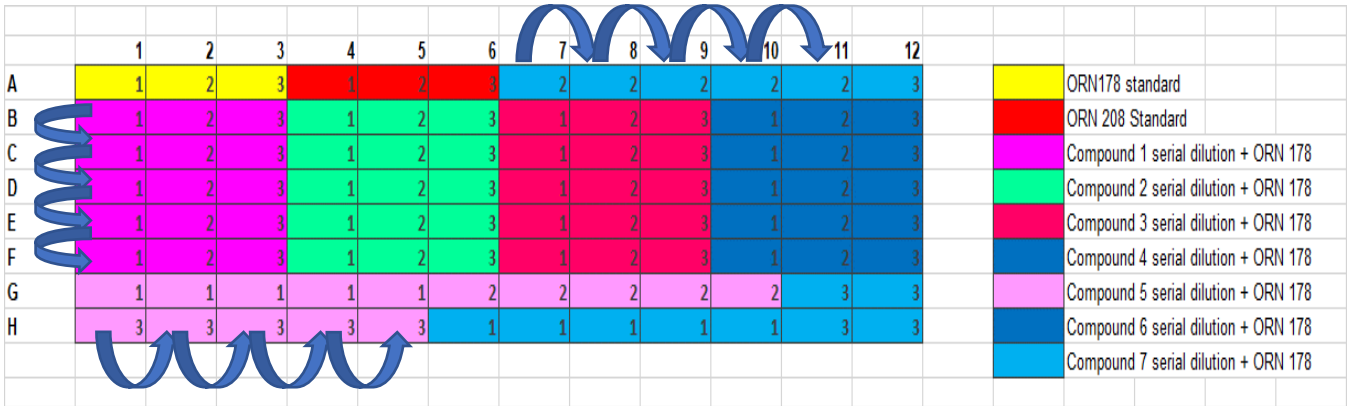

Figure 2: 96-Well Plate Set-Up

Allow to incubate for 45 min, shaking at room temperature. Suck out bacteria and wash twice with 100µL DPBS. Add 100µL of LB to each well.

Cover plate and place in EPOCH 2 plate reader with continuous shaking (double orbital) at 37°C measuring at OD_{600} in 15 min intervals for 20 hours. Alternatively, place in shaking incubator at 37°C and measure OD_{600} after 2.5 hours of growth.

Day 4 → Export data into excel, save and analyze.