HOST-PATHOGEN INTERACTIONS LAB
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TITLE: EXAMINING IPTG-INDUCED EFFECTOR EXPRESSION LEVELS IN EPEC COMPLEMENTED STRAINS BY WESTERN BLOTTING

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1. Streak a plate for EPEC strain containing the desired effector protein encoding plasmid (according to the EPEC strains inventory file) with appropriate antibiotics and incubate O/N @ 37°C. IMPORTANTLY: These EPEC strains are typically mutants deleted in a specific effector, complemented with a plasmid encoding an effector that is typically tagged (HA, FLAG, SBP etc) – e.g. EPEC-*espH* + EspH-SBP. Therefore, the control experiment for these experiments will be the EPEC bearing the deleted gene; e.g. EPEC-*espH*.
2. **LB starter culture**: Pick a single colony from the plate in step-1 and inoculate into a snap cap tube with 3mL LB+ appropriate antibiotics. **Incubate O/N in 37°C without shaking**. Include a tube with LB and no bacteria as control for unwanted infection- **if this control is turbid after O/N culturing, then your LB is considered contaminated- discard the experiment!**
3. **Activation tube**: Aliquot 5 ml of an appropriate tissue culture medium (usually HDMEM) in 50 ml centrifuge tube, the cap should be loosely closed and place it in CO_2 incubator for overnight. **Media should not contain any antibiotics and serum.**
4. Following day, inoculate 100 µl of the **LB starter culture** into the **activation tube** containing 5 mL of the appropriate tissue culture medium (1:50 ratio; LB starter culture: Tissue culture media, typically DMEM or MEM).
5. Incubate the culture from **Step 4** in 37°C + 5% CO_2 (without shaking) for 3 hours. Pay attention to keep the test tube cap loose to allow the entry of CO2; After 2.5 hrs. add the appropriate concentration of IPTG and place it back into the incubator for another half an hour. Use **freshly prepared** IPTG stock diluted to **0, 0.05, 0.1, 0.2, 0.25, 0.5** mM into the DMEM/activated bacteria mixture. There is no need to add IPTG to the deletion mutant.

6. After the incubation is over, mix the tube and immediately take 1 ml of the culture into 1.5 ml micro-centrifuge tube, spin at 6000 rpm for 2 mins.
7. Discard the supernatant and resuspend the pellet in 200 ul 1X SDS-PAGE sample buffer.
8. Heat 95 °C for 10 min, spin-down maximum speed for 1 min.
9. Take sup and load 20 µl per sample well onto a gel (since the effectors are small – up to 20 kDa – 13% gels are typically used) and analyze by Western blotting.

   Gels should be loaded in the following order:

   1. Mw markers
   2. EPEC-*espH*
   3. EPEC-*espH*+EspH-SBP (0 mM IPTG)
   4. EPEC-*espH*+EspH-SBP (0.05 mM IPTG)
   5. EPEC-*espH*+EspH-SBP (0.1 mM IPTG)
   6. EPEC-*espH*+EspH-SBP (0.2 mM IPTG) or (0.25 mM IPTG)
   7. EPEC-*espH*+EspH-SBP (0.4 mM IPTG) or (0.5 mM IPTG)