Interaction Between NaCl and Insecticide

Media Preparation

Follow Thorpe Lab standard protocol. To make 500ml yeast, peptone, dextrose (YPD) media:

**350ml sterile H2O**

**5g Yeast Extract**

**10g Peptone**

**100ml H2O**

**Once mixed autoclave**

**After autoclaving add 50ml of filter sterilised glucose**

Adding the glucose afterwards avoids the glucose degradation during autoclaving, which leads to variable growth mediums.

Yeast Overnight Culture

For replicates performed on different days to be comparable yeast cells have to be harvested from the same growth phase. Cells will be harvested from overnight cultures in mid-log phase, which is 0.4-0.6 OD on the Thorpelab spectrophotometer. The stock wild-type yeast strain, BY4741, is stored as colonies on agar plates at 4oC. Innoculate 5ml of YPD culture with 1 yeast colony. Mix the cells and perform x5 serial dilution five times (i.e. 5x, 25x, 125x, 625x and 3125x dilutions). Place these overnight cultures in the orbital shaking incubator (225rpm) overnight at 30oC. **Begin and end process at the same time every day (1100, 1300).** By the following morning, I expect that one of the serial dilutions will be in log phase (between 0.4-0.6 OD) (x125). Use log phase overnight culture for subsequent experiments. To increase cover also perform x10, x20, x50, x75 and x100 dilutions. If an overnight culture isn’t within the 0.4-0.6 OD range, then the experiment has to start again. **Label each tube with dilution factor and date**

x1 = 10ml media + 2 colonies

x5 = 8ml media + 2ml x1

x10 = 2.5ml media + 2.5ml x5

x20 = 3.75ml media + 1.25ml x5

x25 = 8ml media + 2ml x5

x50 = 2.5ml media + 2.5ml x25

x75 = 3.35ml media + 1.65ml x25

x100 = 3.75ml media + 1.25ml x25

x125 = 4ml media + 1ml x25

Dilute this overnight culture down to 4ml 0.05 OD solution.

* Continuous dependent variable of total growth (AUC).
* Categorical independent variable (treatment). Treatments are levels in a factor.
* Two insecticides, one concentration (5mM), with and without salt. Also, no salt (control) and salt.
* Salt is 0.5M NaCl (0.4M final well conc).
* Ten levels in factor
  1. salt
  2. no salt
  3. acet
  4. imi
  5. cloth
  6. flu
  7. acet+salt
  8. imi+salt
  9. cloth+salt
  10. flu+salt
* With 9 replicates each the whole expt can fit all on one plate.
* Do three blanks with media and three blanks with NaCl media.

Make 2 x 1ml 6.25mM stock solution for each insecticide

* 12.5µl master stock + 987.5µl media (x2)

Make 2 x 1ml 6.25mM NaCl stock solution for each insecticide

* 12.5µl master stock + 987.5µl NaCl media (x2)

For 5mM final well concentration

* 160µl 6.25mM stock + 40µl yeast culture

For control

* 158µl media + 2µl DMSO + 40µl yeast culture

For control+salt

* 158µl NaCl media + 2µl DMSO + 40µl yeast culture

Final NaCl conc 0.4M

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| acet |  |  |  |  |  |  |  |  | acet + salt |  |  |
| imi + salt |  |  |  |  |  |  |  |  |  |  |  |
| flu + salt |  |  |  |  |  |  |  |  |  |  |  |
| cloth + salt |  |  |  |  |  |  |  |  | imi |  |  |
| control + salt |  |  |  |  |  |  |  |  |  |  |  |
| flu |  |  |  |  |  |  |  |  |  |  |  |
| cloth |  |  |  |  |  |  |  |  | blank |  |  |
| control |  |  |  |  |  |  |  |  |  |  |  |

Day Plan

Monday

Start overnight culture.

Check media isn’t contaminated. If it is, make some more or use bottle that isn’t contaminated up.

Halo Assay

PDR1/3 knockout discuss with Cinzia.

Tuesday

Check overnight culture.

Place master solution and media in incubator to warm.

Turn on heatblock.

Set up sterile hood.

Start expt.

Once stock solution, media and DMSO added go downstairs and make 0.05OD yeast culture.

Measure OD of overnight culture.

Take 0.4-0.6 OD culture back upstairs and finish expt.

Need 5ml of 0.05OD stock.

Spectrophotometer (4pm free).

Home.