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 8ml 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).
* Five levels in factor
  1. control
  2. acet
  3. imi
  4. cloth
  5. flu
* Binary Option – Salt or No 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.

For 5mM final well concentration

* 158µl media + 2µl 0.5M insecticide stock + 40µl yeast culture

For 5µM final well concentration

* Make 0.2ml of 6.25mM (x9)
  + 2.5µl 0.5M stock + 197.5µl media
* Make 0.1ml of 0.625µM stock (x9)
  + 10µl 6.25mM stock + 90µl media
* 1.6µl 0.625mM stock + 156.4µl media + 40µl yeast culture

For control

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

Repeat for both Salt and No Salt condition

5µM stock although longer avoids potential pseudoreplication if one 6.25mM stock was used for all wells.

Final NaCl conc 0.4M

Day 1:

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

Day 2:

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