Detailed Method for Yeast Growth Rate Assay

1. Prepare Media:

Follow Thorpe Lab standard protocol.

To make 500ml yeast, peptone, dextrose (YPD) media

350ml sterile H2O

5g Yeast Extract

10g Peptone

50ml 20% Glucose (w/v)

100ml H2O

Once mixed autoclave.

1. Yeast Overnight Culture:

Remove colony stock from 4oC cold room. Streaked onto agar plate in January by Peter. Viable for approximately a year. Next to a flame measure 5ml of YPD into a Falcon tube. Using a sterilised stick (pass through flame a few times too) insert 1 yeast colony into the media. Vortex and place in orbital shaking incubator (250rpm?) overnight at 30oC. Begin and end process at the same time every day (2pm, 10am). This is the standard method followed by Thorpe Lab (apart from the timings). Yeast reach stationary phase. Maybe an additional step here to resume growht. Dilution at 10am. Leave for some hours and take measurement. Harvest cells between 0.4-0.6. Work out what dilution and growth time is needed to reach 0.4-0.6.

1. Insecticide Stock Solutions:

0.5M stock solutions are initially created. The highest concentration tested will be 5mM. Consequently, there will be a 1% final insecticide solvent concentration. To allow for comparison this solvent concentration has to be maintained for all insecticide concentrations. Insecticide solubility figures aren’t available for one solvent. Instead they are split across DMSO and methanol, which are both miscible in water. To control for this variation 1% of each solvent will be present for each data point.

1. Effects of 1% DMSO and 1% Methanol on BY4741 Growth:

* Measure OD of overnight culture. Dilute with media if outside linear range of spectrophotometer (0.1-1). Once in linear range calculate dilution required for final OD of 0.05. This will determine the volume of yeast culture added to each well.
* Treatments (5 replicates of each):
  + Control = yeast + media (n=5).
  + Treatment 1 = yeast + media + 1% DMSO (n=5).
  + Treatment 2 = yeast + media + 1% methanol (n=5).
  + Treatment 3 = yeast + media + 1% methanol + 1% DMSO (n=5).
* 96-well plate
* Make each replicate separately directly into well. For example, if OD of yeast culture was 0.5 then 1µL methanol + 10µL yeast culture + 89µL media for final volume of 100µL and 1% methanol concentration.
* Randomise plate using R script to control for pipetting error, temperature fluctuations etc.
* Add media first, then stressors and mix thoroughly, then finally yeast stock.
* Quantify growth rate for 16h.
* Collect results the next day and analyse.
* Calculate log phase gradient (growth rate) for each treatment.

1. Effects of Insecticide on BY4741 Growth:

* Follow similar procedure to 4. n=3 instead of 5.
* Room temperature.
* 96 well plate. 100µL final volume each well.
* Concentrations 0µM, 10µM, 100µM, 1mM, 5mM for each of the following 13 insecticides. 195 wells in total.
* Instead of making replicate directly into well, make 500µL in Eppendorf first then aliquot 100µL into well. Why? Wasteful but most accurate. If smaller volumes are produced multiples of 0.05µL will have to be pipetted. Don’t think this is an option.
* Remember to note and label well allocation for each insecticide.
* 1% for both solvents.
* Yeast culture with 0.5OD.

1. Make 200μl of 20mM working solution from 0.5M stock solution.

* 8μl 0.5M stock solution + 192μl media.

2. Make 500μl of 5mM final solution from 20mM working solution.

* + 125μl 20mM working solution + 320μl media + 50μl yeast culture + 5µL methanol.

3. Make 500μl of 1mM final solution from 20mM working solution.

* 25μl 20mM working solution + 50μl yeast culture + 4μl DMSO + 416μl media + 5µL methanol.

4. Make 500μl of 100μM final solution from 20mM working solution.

* 2.5μl 20mM working solution + 50μl yeast culture + 4.9μl DMSO + 437.6μl media + 5µL methanol.

5. At this stage make 100μl of 100μM working solution from 20mM working solution.

* 0.5μl 20mM working solution + 499.5μl media.

6. Make 500μl of 10μM final solution from 100μM working solution.

* 50μl 100μM working solution + 50μl yeast culture + 5μl DMSO + 390μl media + 5µL methanol.

7. Make 500μl of 0nM final solution (control).

* 50μl yeast culture + 5μl DMSO + 440μl media + 5µL methanol.

8. Make double negative control.

* 50μl yeast culture + 450μl media.

9. Add media blanks.

* 500µL media.