Troubleshooting Growth Assay

Issues.

* Exponential growth only lasts for an extremely short amount of time.
* Lowering the initial yeast concentration doesn’t extend the exponential phase.
* Initial yeast concentration dilutions didn’t grow to same cell density at stationary phase (﻿stationary-phase OD increments).
* No lag phase. Is this because there is no change in environment when moving from media to media?

Solutions.

* Use a parafilm cover to remove any evaporation effects. Results look like all dilutions hit stationary phase at same time. Could be due to evaporation. Same nutrient amount in all wells so wells with lower initial concentration of yeast should run out of nutrients later than wells with higher initial concentration.
* Redo dilution expt with a parafilm cover as results are extremely unexpected.
* Shake more!
* Try and introduce a lag phase by resuspending yeast cells in water. Then they will have to acclimate to a new environment (media), which should introduce a lag phase. Creates problems for RNA-seq expt. Would have to concentrate in water and then resuspend in media. Don’t want to introduce shock.
* Grow cells to stationary phase, then dilute, wait a certain amount of time and treat. This isn’t advisable because a myriad of transcriptomic changes related to metabolism occur during stationary phase. Also, the cell wall thickens. Individual cells will exhibit variation in their transition from stationary to log phase. Therefore, when the treatment is applied the cells will be in a transition from stationary to log and be heterogeneous. Want all cells to be in one phase (log)
* to ensure a uniform transcriptomic background. Can’t treat stationary phase because they will be more resistant to toxicants due to thicker cell wall, cell cycle arrest etc.

Yeast cells have to be in the log phase for both the growth assay and RNA-seq experiment. This is because pesticide effects may differ between different growth phases and the aim of this experiment is to associate particular gene expression changes with pesticide induced growth inhibition. Pesticide induced transcriptomic alterations may vary between growth phases, as may pesticide induced growth inhibition. Therefore, if the two experiments are carried out in two different growth phases, the pesticide driven transcriptomic alterations, and associated perturbed biological processes and metabolic functions, can’t be causally related to the pesticide induced growth inhibition. Furthermore, the objective of this expt is to reveal the genes differentially expressed when a pesticide concentration is applied that results in 20% growth inhibition (or lowest observed effect concentration). The concentration required for 20% growth inhibition is likely different for cells in different phases of growth.

Progress (31/07/2020)

* After adding a parafilm cover and increasing the shaking time from 20s to 60s the growth curves returned the expected result of all reaching the same final OD, with more diluted starting concentrations being shifted to the right. However, now the graphs are choppy. Consequently, the maximum gradient windows are misleading.
* Could potentially switch round the the shaking and kinetic window times.
* Currently, the machine settings are: settling time – 0.5s, No. of cycles – 195, Measurement start time – 0s, No. of flashes per well and cycle – 22, cycle time – 300, shake mode – double orbital, frequency – 200rpm, shake time – 60s, shake before each cycle.
* Switch the shake time to 300s and the kinetic window to 60s. Then there would be more shaking, which is closer to optimal conditions (continuous shaking).
* Currently, I don’t think shake time is counted towards time. Have to correct for that.

Progress (04/08/31)

* Shake time is taken into account for cycle windows.
* The maximum number of wells that can be used at once without using the well perimeter is 60.
* This happens to be (10 insecticides \* 5) + 5 blanks + 5 controls = 60 wells.
* For a 300s cycle time the maximum shake time is 272s. This is because it takes 28s to take readings in 60 wells.
* The measurement time is dependent on how many flashes per well and how many wells.
* For the control growth assay and solvent growth assay set the plate layout to 60 wells because this will be the layout for the subsequent pesticide growth assay.
* Hoping to see classical growth curves now the plates are being shaken for 90% of the 16h total time.
* Going to try these machine settings (FLUOstar Omega): 600nm, settling time – 0s, No. of cycles – 193, measurement start time – 0, No. of flashes per well and cycle – 22, cycle time – 300s, plate layout – 60 wells (36 perimeter wells empty), shake mode – double orbital, frequency – 200rpm, shake time – 272s, shake before each cycle. Minimum cycle time – 300s, total measurement time 16h05 (1 shake before first cycle for approx. 5 mins).
* To introduce a lag phase, I could switch from YPD media to synthetic media for the actual expt. Have to see how this interacts with insecticides. Due to there being a larger change in the environment this could elicit a lag phase.
* Why are the graphs choppy? 5 minute kinetic windows.
* Don’t have to set the layout to 60 each time. Just set the shaking to 272s. This way the conditions are exactly the same as when using 60 wells.
* I think attaching the parafilm cover interferes with the OD measurements. Blank readings don't stabilise until around the 1 hour mark. This creates negative readings at the beginning of the run that produce NaN when ln(). Also, dividing by t~0~ no longer has the desired effect as t~0~ has been inflated, causing negative readings once ln(). If geom\_smooth is used instead of geom\_line it hides a multitude of sins.

Progress (12/08/31)

* Upped volume to 200µl and started with a theoretical 0.01OD yeast conc. Have to use a parafilm cover to avoid contamination.
* If after this there are still a very small amount of timepoint windows associated with ≥95% maximum growth rate, I’ll have to think of another way to define the exponential phase. (Biesiadecka et al., 2020) defined the exponential phase as between 0.12-0.4 from visual inspection and only used figures in that range to calculate the growth rate. Could follow a similar method.
* All was well. I dropped the timepoint window association to ≥80% and allowed for a gap of ≤5 windows to occur in order to select windows associated with the exponential phase.

Progress (25/08/2020)

* 5mM Thiacloprid and Cypermethrin appear to crystallise out.