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Candidate Diploid Yeast Mutants in Solid Media

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Introduction:

Diploid yeasts' similarities with animal cells provides researchers with an important method of understanding cellular function and communication of eukaryotic cells. Yeast growth and a kind of asexual reproduction, sporulation, in biofilms has many commercial and medical implications. Biofilms form on medical devices and cause resistant infections. These infections are difficult to treat with antibiotics because of the structure and nature of biofims. This is a particularly challenging problem in patients that have artificial implants such as heart valves, prosthetic joints and even long term catheters and ports are highly susceptible to biofilm formation. Because of the difficulty at treating these infections, the most common result is the removal and replacement of the artificial equipment. The purpose of this experiment in Dr. Honigberg's laboratory at the University of Missouri in Kansas City is to isolate homozygous diploid yeast mutants that have defective sporulation in solid media and not in liquid media. Solid media sporulation is important over liquid media to select for mutants that are involved in biofilm formation on a hard surface. The results of this experiment were compared to two other laboratory's yeast sporulation in liquid media to determine which mutants sporulate in liquid media that do not sporulate in solid media. Three plates of 96 mutants were screened for defective sporulation out of 5000 mutants. These mutants' deleted genes are likely involved in solid media sporulation and communication.

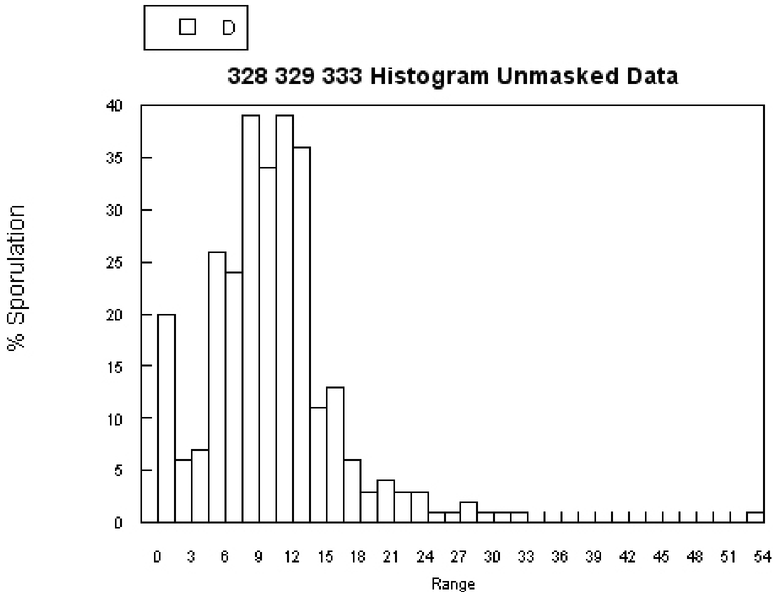
Methods:

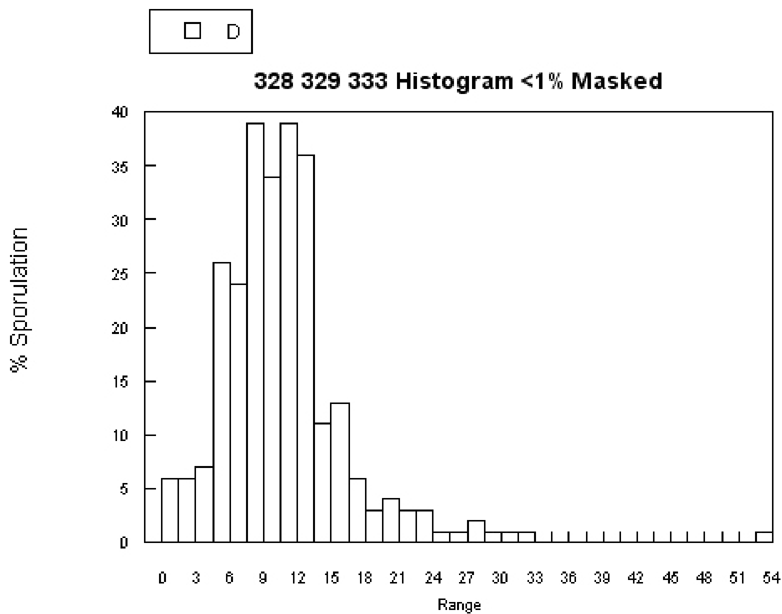
First, the plates that were to be studied in the 5000 mutants were removed from the laboratory's library in a freezer of -80° C and allowed to warm to room temperature. A plate with 96 wells was inoculated with 200µL of YPDA + tet. After the frozen plate was defrosted, a frogger dipped in ethanol and flamed was used to inoculate the wells. The well plate was then grown for 40 hours at 30° C. After the incubation time it was removed and inoculated to a new well plate that had 200µL of YPA + tet. This plate was then returned to the 30° C incubator for 72 hours. The YPDA well plates were then spun down

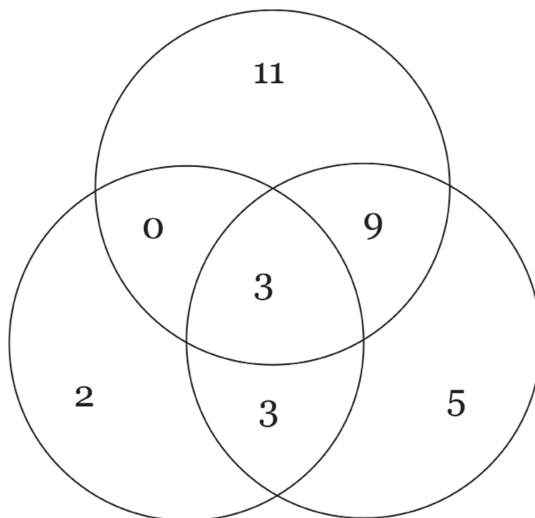
for 4 minutes at 2750 rpm. The 12 channel electronic pipettman was used to remove the liquid, leaving the cells in the bottom of the well. The cells were resuspended in 200µL of 50% glycerol. They were then covered with a sterile seal and restocked into the mutant library in the freezer.

After 72 hours of growth, the plates were removed from the incubator and the flamed frogger was used to spot onto three SPO+ye+glucose+aa plates. They were double wrapped with Parafilm and place in the 30° C incubator for two weeks. The plates were removed, and using a sterile pipette tip, the colonies were individually scraped off the media and placed into tubes with 200µL of sterile water. The mutants were then all counted individually under a microscope for sporulation. Approximately 200-250 cells were counted for each set of mutants. They were counted in three categories: tetrad (4), dyad (2), and vegetative (1) cells. These values were used to calculate the percent sporulation and recorded.

Results:




D
328 329 333 Histogram <1% Masked
% Sporulation
40
35
30
25
20
15
10
5
0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54
Range


23 Mutants
11
0
9
3
2
3
5
Enyenihi 8 Mutants
Deutschbauer 19 Mutants

The results of this experiment showed 23 mutants among the 288 tested. The Venn diagram above shows the overlap with Enyenihi and Deutschbauer in their experiments with sporulation mutants in liquid media. If a mutant was defective in liquid and in solid media it is not considered a candidate for solid media sporulation deficiencies. Twelve of Deutschbauer's and three of Enyenihi's had overlapping mutants. There were 11 mutants I found that were not identified as mutants by the other two laboratories liquid media sporulation experiments. Of these eleven, two did not show any growth, thus negating an opportunity to observe the sporulation amount, leaving nine candidates.

Unmasked Data: Points = 282.00 Mean = 10.185 Median = 9.80
Std Deviation 6.1259

Below 1% Masked Data: Points = 268.00 Mean = 10.704 Median = 10.000
Std Deviation 5.8352

The threshold for mutant determination was 1.5 standard deviation.
10.704-(1.5*5.835)=1.95

ORF	Record		Plate	row	col	
YFR010W	35689	1	06_1	328	D	6
YIR037W	35972	1	00_3	328	H	12
YKR070W	35986	1	00_3	329	A	10
YKR082W	35998	1	00_3	329	B	7
YMR062C	36195	1	00_6	329	B	9
YOL129W	36279	1	00_6	329	H	6
YOL138C	36288	1	00_6	329	H	11
YLR447C	36051	1	00_4	333	F	8
YMR060C	36069	1	00_4	333	G	10

Discussion:

The description of the functions of the nine candidate genes was interesting. Three of the genes had unknown function. YOL138C and YKRO7W are described as proteins of unknown function and YOL129W is described as a vacuolar membrane protein of unknown function. YFR010W is involved in protein deubiquination. YIR037W is a thiol perioxidase that responds to oxidative stress. YKR082W functions in DNA metabolic processes. YMR062 functions in the arginine biosynthic process. YLR447C is involved in vacuolar transport. YMR060C is a component of the outer mitochondrial membrane.

All of these genes have some effect on the sporulation of the yeast on solid media. It may be due to the lack of functioning machinery needed to go through the process of sporulation or defective

structural components for sporulation. It could also be caused by the lack of signaling or disrupted signaling. In liquid media there is approximately equal availability of nutrients and oxygen to all cells. However, in a solid colony, there is a striation of nutrients and of space. The inequality of location within the colony of solid media makes the cell-to-cell interactions more complex. Understanding the genes that regulate sporulation could help to understand the most basic pre-tissue interactions and how location affects a cells behavior.
Conclusion:

In conclusion, the results of this experiment after a comparison with two other laboratory's yeast sporulation in liquid media 9 mutants were found that did not sporulate on solid media. These mutants are the best candidates for father testing to determine the role of the gene deletion in sporulation. The mutants' deleted genes are likely involved in solid media sporulation and communication. After more testing, it may be possible to determine the specific role of each gene in sporulation in a solid media colony further understanding the complex communication between neighboring cells and their environment in biofims.

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