Grow yeast and refresh

Purpose:

Growing yeast to an appropriate amount that we can use for experiment

Material:

- YPD (yeast extract, peptone, dextrose)
- 0.2% Adenine (working at 0.002%)

Procedure:

Grow yeast

1. Put 3ml YPD into glass tube, and add in 30 μ l adenine.
2. Use wood stick to pick some yeast from the YPD plate, stir into medium.
3. Incubate at 30^{o}C overnight.

Refresh

1. Put 800 μ l yeast in cuvette and test in OD600, the number should be over 1.
2. Dilute yeast with YPD into OD600 0.05.
3. Incubate yeast in 30^{o}C for 4 hours.
4. Put 800 μ l yeast in cuvette and test in OD600, the number should between 0.3-0.5.
5. Now you have a tube of healthy yeast to do the experiment.

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If yeast will be harmed by Alcian Blue 20170722

Prupose:

We want to find a way to make yeast stick more specific on the ink particle. We made a hypothesis that ink will bind to ink, so we want to make yeast coated with Alcian blue and after that we will pour yeast onto paper to see if it can binding with ink. However, the first thing we want to make sure is that if Alcian blue will harm the yeast or not.

Material:

- Liquid YPD
- Adenine
- HCL
- Alcian blue (0.05%, 0.1%, 0.5%, 0.8%, 1%)
- NaCl
- YPD plate

Procedure:

1. 1. Liquid culture SCSY 79 strain in 3 ml YPD+30 μl Adenine at 30° C overnight.
2. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
3. Dilute the OD_{600} to 0.05 with 10 ml YPD and put refresh sample at 30° C for 3.5-4 hours. The new OD600 should be between 0.3-0.5.
4. Centrifuge 2 minutes 2000 rpm, discard supernatant
5. Use 14 ml ddH2O to resuspend the pellet and separate into seven tubes.
6. Centrifuge 2 minutes 2000 rpm, discard supernatant
7. Labeled tubes from 1-7, the first 2 tubes is the control
8. Add 7 ml ddH_2O in tube 1, add 7 ml NaCl to resuspend the pellet
9. Add 2ml ddH_2O in tube 3.4.5.6.7 to resuspend the pellet
10. Add 2 ml 0.05% stain in tube 3, 2 ml 0.1% stain in tube 4, 2ml 0.5% stain in tube 5, 0.8% stain in tube 6, 2 ml 1% stain in tube 6.
11. Incubate for 15 mins
12. Centrifuge 2 minutes 2000 rpm, discard supernatant.
13. Use 7 ml HCL to wash pellet twice.
14. Use 2ml YPD resuspend the pellet.
15. Take 200 μl yeast and plating on YPD plate.

Result:

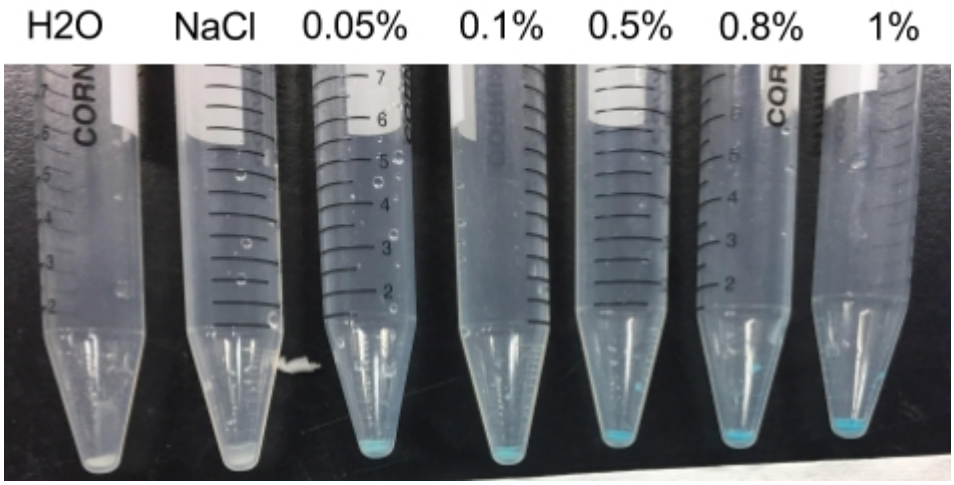

Figure 1. stain coated on the yeast

The figure shows the result of yeast treated with different condition. From left to right is H2O, NaCl, 0.05% of stain, 0.1% of stain, 0.5% of stain, 0.8% of stain and 1% of stain.

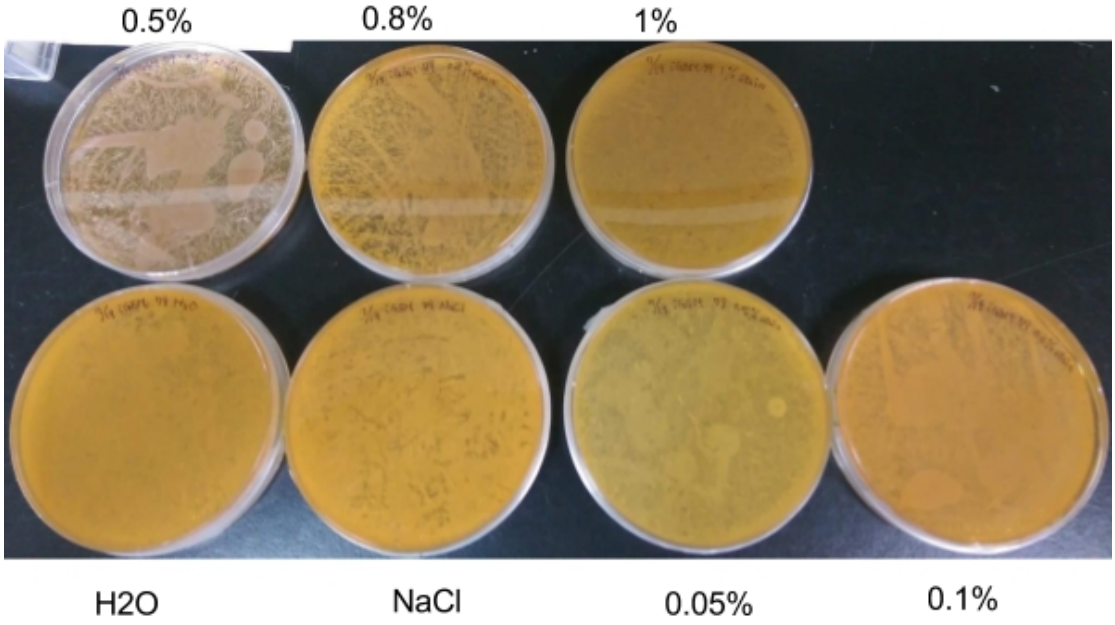

Figure 2. What concentration of stain will hurt yeast

This figure shows the plate result of yeast treated with different condition. Top three from left to right is yeast treated with 0.5% of stain, 0.8% of stain and 1% of stain. Bottom four from left to right is yeast treated with H2O, NaCl, 0.05% of stain, 0.1%

of stain.

Discussion:

Yeast treated with different condition all grow full on the plate. It shows that this stain won't hurt yeast. However, we think it might has a possibility that using YPD to resuspend stained yeast made yeast turn stronger again. It might also be possible that we plating too much yeast, so we can't tell the difference. Look under microscope, we can see stained yeast was coated with a blue circle compare with the control.

After wash step you can see clearly the yeast was coated with blue and pellet's color will go deeper while the concentration was higher. From the color of pellet we can know that yeast was coated with Alcian blue.

If yeast will be harmed by Alcian Blue 20170727

Prupose:

From last experience, none of the percentage of stain would harm the yeast, so we change the percentage to 2%, 4%, 8% and 16%. We want to know the limitation of yeast that can tolerate with Alcian blue.

Material:

- Liquid YPD
- Adenine
- HCL
- Alcian blue (2%, 4%, 8%, 16%,)
- NaCl
- YPD plate

Procedure:

16. 1. Liquid culture SCSY 79 strain in 3 ml YPD+30 μ l Adenine at 30° C overnight.
17. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
18. Dilute the OD_{600} to 0.05 with 10 ml YPD and put refresh sample at 30° C for 3.5-4 hours. The new OD600 should be between 0.3-0.5.
19. Centrifuge 2 minutes 2000 rpm, discard supernatant
20. Use 12 ml ddH2O to resuspend the pellet and separate into six tubes.
21. Centrifuge 2 minutes 2000 rpm, discard supernatant
22. Labeled tubes from 1-6, the first 2 tubes is the control
23. Add 7 ml ddH_2O in tube 1, add 7 ml NaCl to resuspend the pellet
24. Add 2ml ddH_2O in tube 3.4.5.6.7 to resuspend the pellet
25. Add 2 ml 2% stain in tube 3, 2 ml 4% stain in tube 4, 2ml 8% stain in tube 5, 16% stain in tube 6
26. Incubate for 15 mins
27. Centrifuge 2 minutes 2000 rpm, discard supernatant.
28. Use 2 ml HCL to wash pellet twice.
29. Use 2ml ddH_2O resuspend the pellet.
30. Take 200 μ l yeast and plating on YPD plate.

Result:

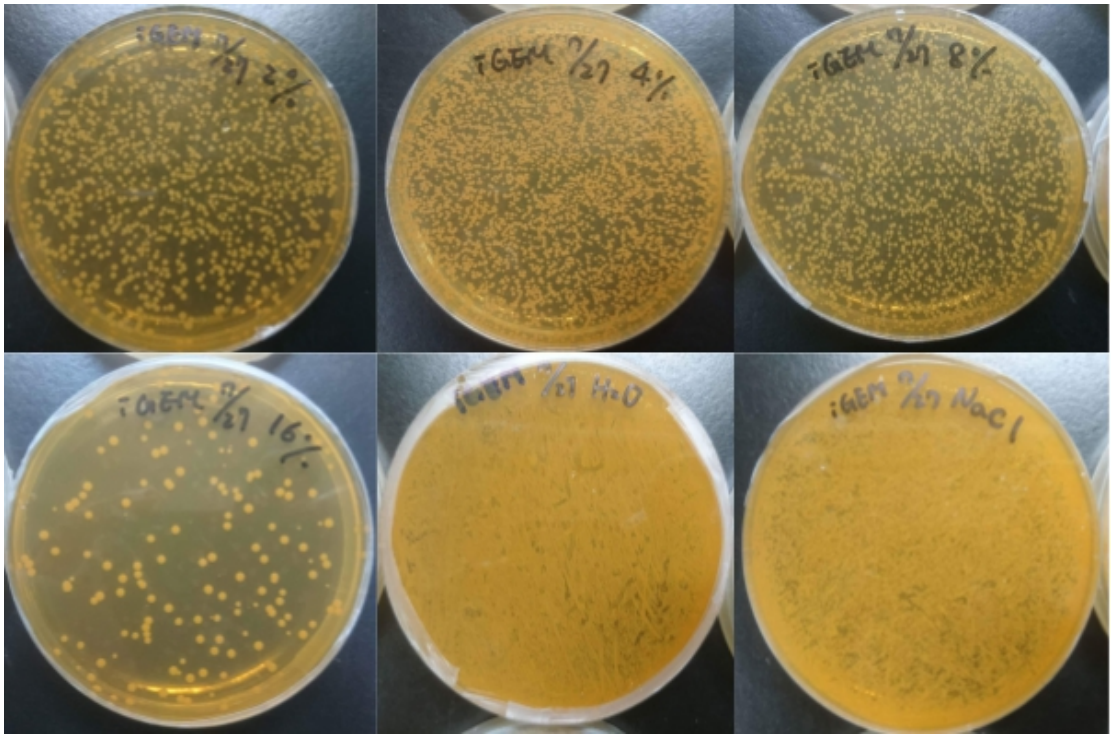

Figure 1. What concentration of stain will hurt yeast

The figure shows that yeast treated with different condition. The percentage is the stain concentration.

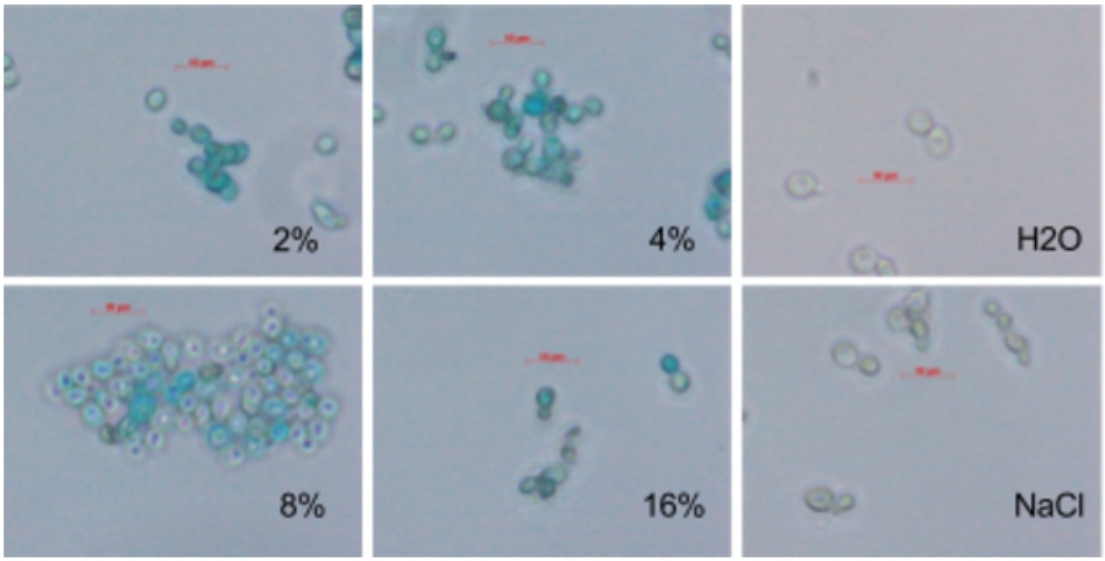

Figure 2. Yeast under microscope

In the figure, yeast is coated with Alcian blue. The percentage on the figure is different stain concentration. Right two picture is the control condition, H2O and

NaCl.

Discussion:

We can see the result in the 9 conditions we build. The answer is positive that Alcian blue will hurt yeast cell. From the plate we can see the colony number will reduce when the stain concentration goes on. Under microscope we can see after 4%, most of yeast's nuclei turns dark blue instead of membrane is stained. The stain shouldn't goes into cell, once it happened means cell is dead. The result shows that Alcian blue will kill the cell and after 4% yeast start to dye. One special things is that 2% is less than 4% even 8%.

If yeast will be harmed by Alcian Blue 20170806

Prupose:

From last experiment we found that stain higher than 4% will kill the yeast. This experiment is want to make sure this test result is correct and decide which concentration of stain we want to use in the future experiment.

Material:

- Liquid YPD
- Adenine
- HCL
- Alcian blue (1%, 2%, 4%)
- NaCl
- YPD plate

Procedure:

31. 1. Liquid culture SCSY 79 strain in 3 ml YPD+30 μ l Adenine at 30° C overnight.
32. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
33. Dilute the OD_{600} to 0.05 with 10 ml YPD and put refresh sample at 30° C for 3.5-4 hours. The new OD600 should be between 0.3-0.5.
34. Centrifuge 2 minutes 2000 rpm, discard supernatant
35. Use 12 ml ddH2O to resuspend the pellet and separate into six tubes.
36. Centrifuge 2 minutes 2000 rpm, discard supernatant
37. Labeled tubes from 1-6, the first 3 tubes is the control
38. Add 7 ml ddH_2O in tube 1, add 7 ml NaCl to resuspend the pellet, add 7 ml HCl to resuspend the pellet
39. Add 2ml ddH_2O in tube 4.5.6 to resuspend the pellet
40. Add 2 ml 1% stain in tube 4, 2 ml 4% stain in tube 5, 2ml 8% stain in tube 6, 16% stain in tube 6
41. Incubate for 15 mins
42. Centrifuge 2 minutes 2000 rpm, discard supernatant.
43. Use 2 ml HCL to wash pellet twice.
44. Use 2ml ddH_2O resuspend the pellet.
45. Do serial dilution, dilute cell to 100 cells/ 200 μ l
46. Put 200 μ l yeast solution to plating.

Result:

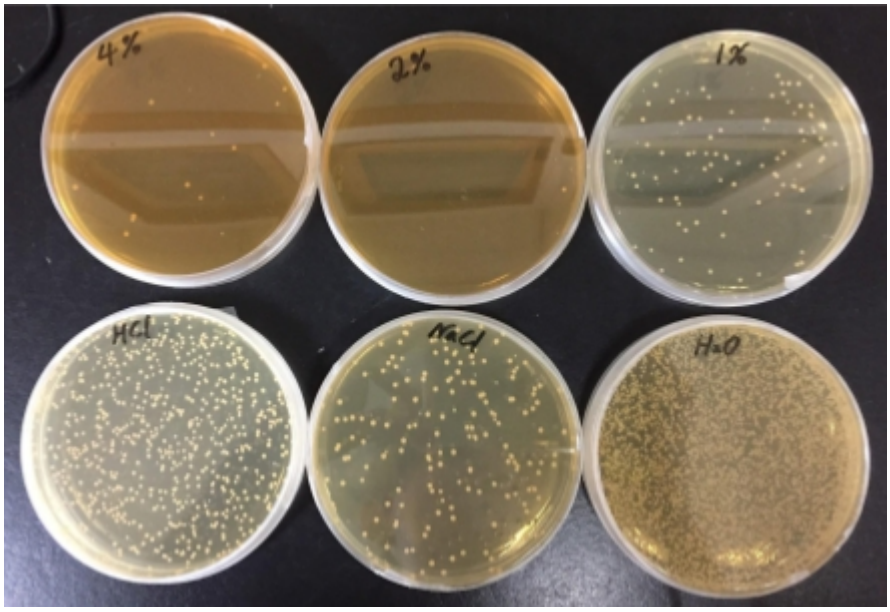

Figure 1. What concentration of stain will hurt yeast

The figure shows that yeast treated with different condition. The percentage is the stain concentration.

Discussion:

To know which buffer makes yeast turn weaker we have a control of H2O NaCl and HCl. NaCl is the staining buffer and HCl is we use to dissolve the stain. The result shows that both of them didn't do much harm to yeast, so we make sure that stain is the reason that yeast turns weaker. Also, we did confirm that if stain concentration is higher than 1% it will kill most of yeast.

If yeast coated with Alcian blue can have a better binding affinity with paper

20170811

Prupose:

From last experiment, we can't tell if yeast can bind on ink or not. This time, we change the paper picture and using different way to plate yeast on the plate. We want to see the significant difference on the ink and paper.

Material:

- Liquid YPD
- Adenine
- HCL
- Alcian blue (1%)
- NaCl
- YPD plate

Procedure:

47. 1. Liquid culture SCSY 79 strain in 3 ml YPD+30 μ l Adenine at 30° C overnight.
48. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
49. Dilute the OD_{600} to 0.05 with 10 ml YPD and put refresh sample at 30° C for 3.5-4 hours. The new OD600 should be between 0.3-0.5.
50. Centrifuge 2 minutes 2000 rpm, discard supernatant
51. Use 8 ml ddH2O to resuspend the pellet and separate into four tubes.
52. Centrifuge 2 minutes 2000 rpm, discard supernatant
53. Labeled tubes from 1-4.
54. Add 7 ml ddH_2O in tube 1 and 2.
55. Add 2ml ddH_2O in tube 3.4 to resuspend the pellet
56. Add 2 ml 1% stain in tube 3.4.
57. Incubate for 15 mins
58. Centrifuge 2 minutes 2000 rpm, discard supernatant.
59. Use 2 ml HCL to wash pellet twice.
60. Use 8ml ddH_2O resuspend the pellet.
61. Put paper and yeast in clean dish
62. Let them work for 30 minutes
63. Use ddH_2O 5ml to wash the paper
64. Put entire paper on the YPD+A plate.

Result:

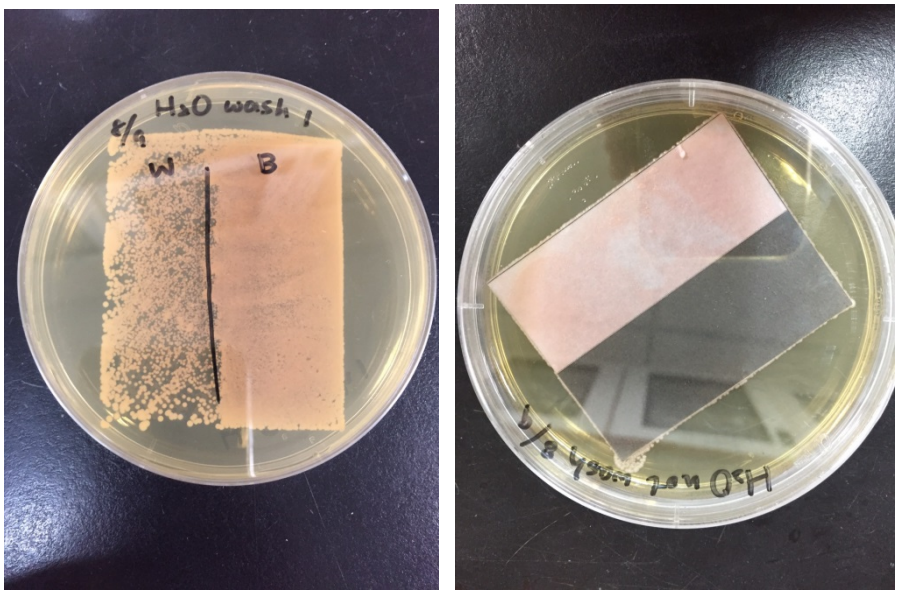

Figure 1. unstained yeast binding with ink

On the left side is the paper without ink and on the right side is paper with ink.

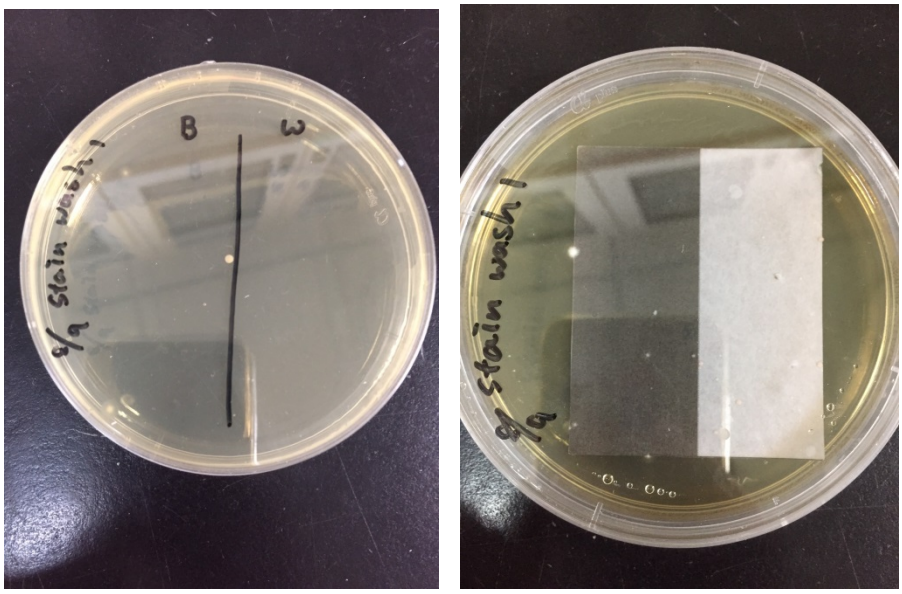

Figure 2. stained yeast binding with ink

On the right side is the paper without ink and on the left side is paper with ink.

Discussion:

From the figure 1 you can see ink will bind to inky part but that is unstained yeast. In figure 2, stained yeast only have one colony and it is even close to the

separation line of black and white. We think 1%of stain might be too strong for the yeast to grow, so after wash and all the treatment most yeast dye. We think even without stain, yeast itself might prefer ink more than paper fiber. In the future work we will keep testing on yeast and ink's binding affinity with more different condition. Also, lowering the stain concentration to stain the yeast and see if it can bind with the ink. We hope to find a best condition that yeast will bind perfectly only on the inky part.

If yeast coated with Alcian blue can have a better binding affinity with paper

20170809 - Yeast-Stain Affinity

Transform xylanase lipase construct in yeast 2017.10.23

Prupose:

put composite plasmid into yeast strain W303 to express xylanase and lipase

Material:

- YPD (yeast extract, Peptone, Dextrose)
- Adenine
- Lithium Acetate
- 40% PEG8000 in 0.1M LiAc
- ssDNA
- ddH_2O
- –LEU plate
- –URA plate
- –LEU, -URA double selection plate

Procedure:

Follow transformation protocol

We use 5mg plasmid DNA to do the transformation.

In negative we using 20 ul ddH_2O

Result:

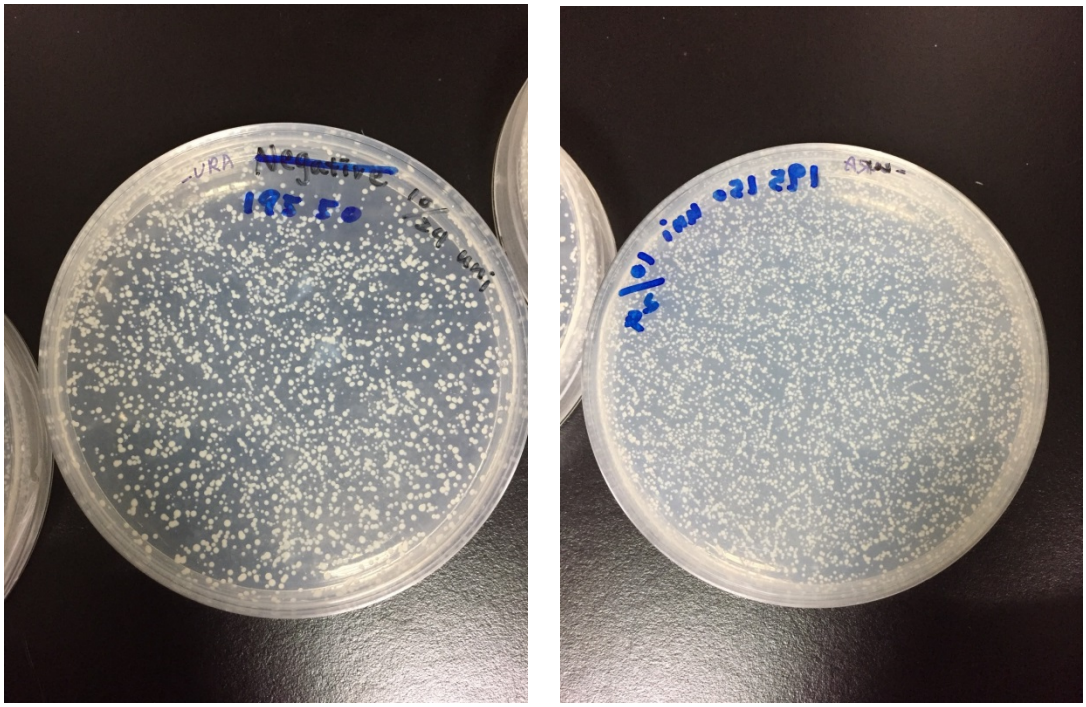

Figure1.2

Transform lipase construct in yeast using –URA selection plate. Figure 1 is using 50 ul to plating figure 2 is using 150 ul to plating.

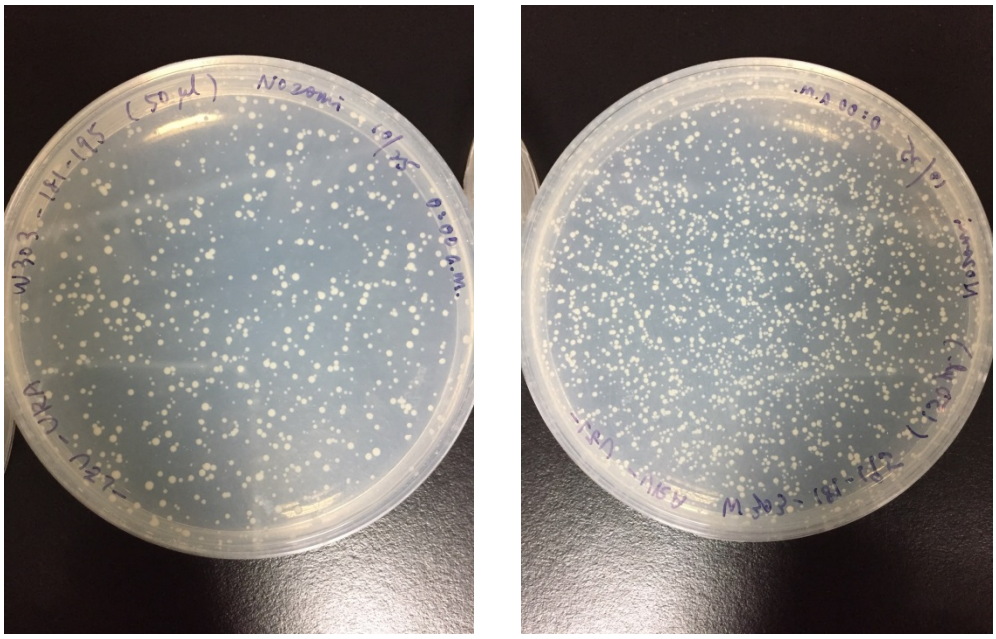

Figure3.4

Transform lipase-xylanase construct in yeast using –URA, -LEU double selection plate. Figure 3 is using 50 ul to plating figure 2 is using 150 ul to plating.

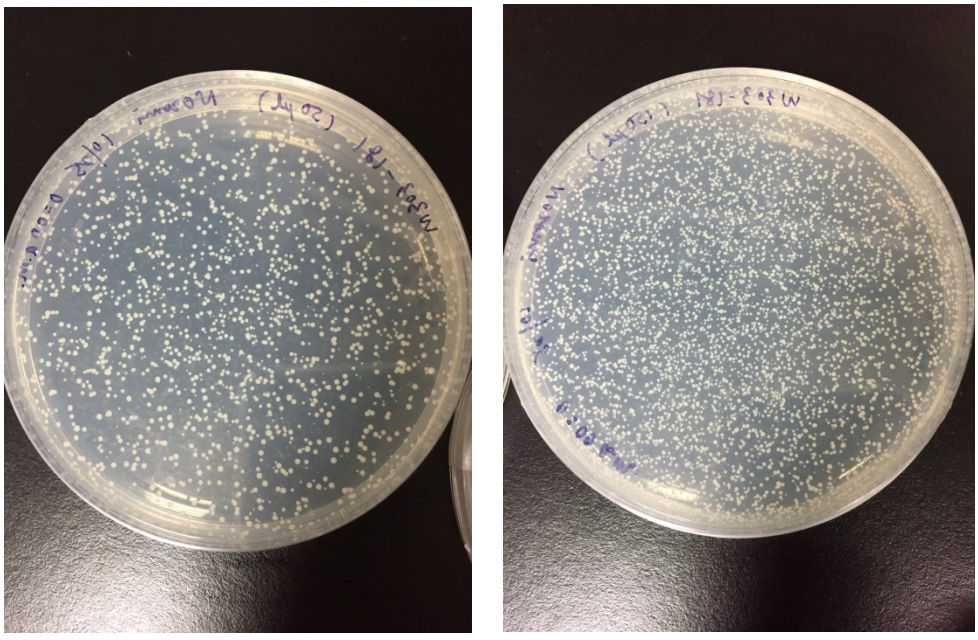

Figure5.6

Transform xylanase construct in yeast using -LEU selection plate. Figure 5 is using 50 ul to plating figure 6 is using 150 ul to plating.

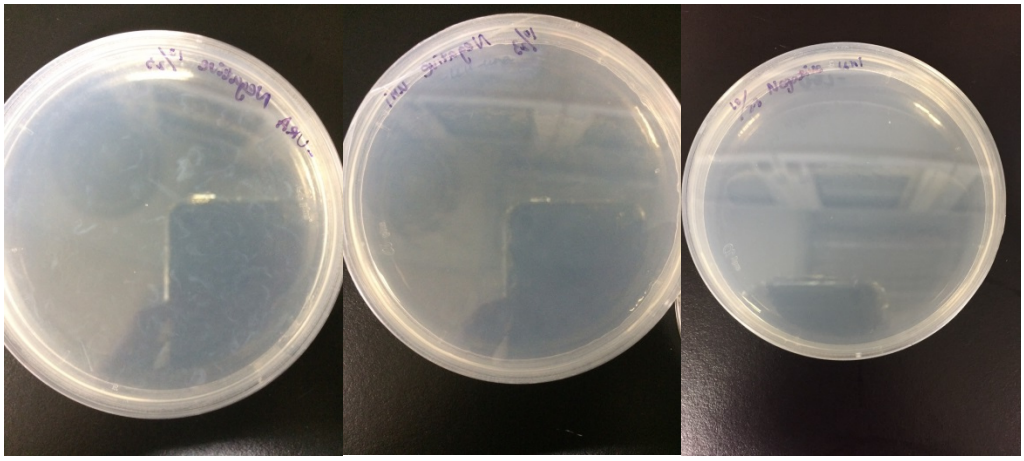

Figure7.8.9

From left to right is the negative control of –URA, -LEU and double selection

Growing yeast in big volume and use alpha factor to induce enzyme

2017/10/27

Purpose:

Grow yeast in big volume to have enough yeast to secret out an adequate amount of enzyme. Use alpha factor to induce W303 to secret out xylanase, and lipase. In this experiment we use W303 as contral.

Material:

- Liquid Selection medium (-LEU)
- Liquid Selection medium (-URA)
- all selection medium
- YPD
- Alpha factor

Procedure:

Follow Growing yeast in big volume and use alpha factor to induce enzyme protocol

Incubate 20 ml for each small volume

Incubate 500 ml for selection medium

Incubate 600 ml for YPD

Result:

Figure1.2.3

Figure1 is the WT induce and noninduced medium. Figure 2 is the lipase induce and non induced medium. Figure 3 is xylanase induced and non induced medium.

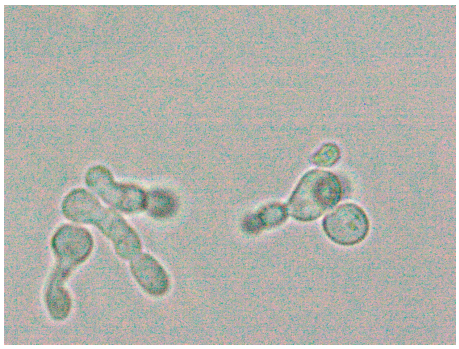
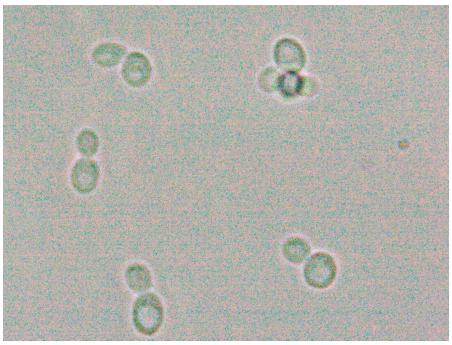

Figure 4.5

Figure 4 is yeast induced by alpha factor. Figure5 is yeast without induced.

How much ml of medium need to be precipitate in order to show band on a SDS-PAGE gel 2017 10 23

Purpose:

To know how much volume of medium need to be precipitate in order to see a clear band on the SDS-PAGE.

Materials:

- 100% TCA
- Acetone
- Tris-base
- 2X Protein sample buffer
- 2-mercaptoethanol
- W303 induced medium

Procedures:

1. Prepare 8 centrifuge tube, add in 50 ml medium in the first 4 tube and 50 ml YPD In last 4.
2. Add 100% TCA, which is a 6% final solution.
3. Mix carefully and incubate on ice for 15 minutes.
4. Spin down for 10 min at 4^{o}at 13,000 g and remove the supernatant.
5. Add 50 ml medium in tube 2,3 and 4. Add 50 ml YPD in tube 6, 7 and 8.
6. Spin down for 10 min at 4^{o}at 13,000 g and remove the supernatant.
7. Add 50 ml medium in tube 3 and 4. Add 50 ml YPD in tube 7 and 8.
8. Spin down for 10 min at 4^{o}at 13,000 g and remove the supernatant.
9. Add 50 ml medium in tube 4. Add 50 ml YPD in tube 8.
10. Spin down for 10 min at 4^{o}at 13,000 g and remove the supernatant.
11. Wash the pellet with 1 ml ice-cold acetone. This helps remove acids and salts.
12. Cut off the end of a pipette tip to make the opening larger, move the sample from centrifuge tube to microcentrifuge tube.

13. Spin down for 10 min at 4^{o} at 13,000 g, remove the supernatant and set for 15 minutes to air dry the pellet.
14. If necessary (too much salt left) redo step 4 and 6.
15. Resuspend pellet in SDS-PAGE protein sample buffer. The TCA pellet can be difficult to resuspend, and it may be necessary to work the pellet into solution with a pipette tip.
16. If the protein sample buffer turns yellow, add 2M Tris-base that has not been adjust for PH, 1 μ l at a time, until it turns blue again. Be sure to add an equal amount of Tris-base to each sample as the extra salt can cause the samples to run differently on the SDS-PAGE gel.
17. Add in 2-mercaptoethanol, which is a 10% final solution.
18. Boil sample at 95^{o} for 5 minutes.
19. Make sure your sample is still blue, if it is not, add in more Tris-base.
20. Ready for loading SDS-PAGE.

Result:

You can see lane after 50 ml precipitation.