Freeze Your Yeast!
Long term storage options for your most precious strains

Tom Schmidlin
Freeze your yeast

• How to successfully freeze your yeast
  – What you need
  – Where to get it

• Why it works
  – Published experimental evidence
  – My tests
How to do it

• Grow your culture to stationary phase
  – Can be straight from a smack pack or tube
• Place culture in refrigerator for 36-72 hours
• Add cold glycerol to 20% final concentration
  – Do not let the culture warm up
• Pour into tubes
• Put tubes in a zip top bag or use parafilm
• Put in the freezer in a frozen, insulated container
What you need and where to get it

• Insulated container
  – Six-pack cooler
  – Small styrofoam cooler
  – Insulated thermos
  – Available from any megastore, ~$10
What you need and where to get it

• Glycerol aka glycerine
  – Available at many pharmacies
  – Used for making soap, lotions, etc

Image from: http://en.wikipedia.org/wiki/Glycerol
What you need and where to get it

• Tubes
  – 1.5 ml Microfuge tubes
  – 15 ml Falcon tubes
  – Anything else that can be sterilized and can handle freezing
  – Available on eBay
    • 1.5 ml tubes 50 for $3
    • 1.5 ml tubes 100 for $4.25
    • 15 ml tubes 50 for $14.95
Why it works – Stationary phase

• Park et al. 1997
• Freeze thaw response is growth phase specific, not controlled by glucose repression
• When cells enter stationary phase, they accumulate glycogen and trehalose, develop thick cell walls, and become thermostolerant
Why it works – cryoprotectants

• Protect cells from damage due to freezing
  – Glycerol
  – Trehalose
  – DMSO
  – Methanol, ethanol

• In some cases it’s not clear how they work
  – Bind to membranes and proteins
  – Prevent formation of large ice crystals
Why it works – trehalose

- Disaccharide made up of two glucose molecules linked via α(1-1) bond

Maltose

Trehalose
Why it works – trehalose

- From Kandror et al. 2004
- Freeze tolerance closely correlates with cellular trehalose content
- Dramatic accumulation of trehalose and induction of trehalose synthesizing enzymes below 10°C
- After 15-20 hours at 4°C, mRNAs of trehalose synthesizing enzymes are up at least 20 fold and maintained at that level for up to 85 hours
- Longer incubation at 4°C results in an increase in cell survival (confirmed by Stoycheva et al. 2007)
- Cells incubated 48h at 4°C accumulated about 15-fold more trehalose compared to cells cultivated at 30°C. incubation for short periods at 4°C increases trehalose only 3-fold.
- Upon return to 30°C, mRNAs, trehalose levels, and tolerance to freezing fall dramatically within minutes
Why it works – other conditions

- Cerrutti *et al.* 2000
  - Specific interactions of trehalose with membranes and/or proteins may help the freeze-drying and vacuum drying processes

- Park *et al.* 1997
  - Freeze-thaw-tolerant yeast strains have higher levels of trehalose
  - High tolerance to freezing during lag phase, low resistance during log phase
  - Trehalose stabilizes the intracellular water structure and cell membranes under stress conditions
  - Cells thawed at 0°C and room temperature did not differ in viability
  - Ice can form intracellularly at high freezing rates
  - External freezing precedes internal freezing, external freezing leads to dehydration and ice formation inside the cell
Strain variability

• Takagi et al. 1997
• Possible to “breed” freeze tolerance
• High gravity strains may tolerate freezing better
Why it works – slow freezing

• Komatsu *et al.* 1987
  • Fast cooling of cells with liquid nitrogen results in damaging of all cellular membranes, including the nuclear one.

• Tanghe *et al.* 2002
  • Rapid osmotically driven efflux of water during freezing reduces intracellular ice crystal formation and resulting cell damage
  • Deletion of *AQY1* and *AQY2* renders yeast more sensitive to freezing, while overexpression improves freeze tolerance
Reviving

• Can pitch whole tube directly into a starter
  – Probably better to start with enriched media
• Can streak to plates and pick a single
  – Helps insure there is no contamination
Plates/Slants/Media

- Raines
- Agar plate: 1-4 weeks
- Agar slant: 0.5-2 years
- Yeast storage media and resuscitation formula

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Dry malt extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Yeast nutrient</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>18.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>to 1000 ml</td>
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</tbody>
</table>
Starters

• Zainasheff
• S.G. between 1.020 and 1.040 or 5-10% malt extract
• 1/4 tsp yeast nutrient per 2 liters
• Boil 15 minutes, cool
• Increase volume by 5-10 times per step
My tests

• Freezing
  – Varied freezing methods and starting temp
  – Plate for viability

• Storage
  – Trial 1 ~ 16 weeks
  – Trial 2 ~ 8 weeks
  – Trial 3 ~ 8 weeks
  – Trial 4 ~ 6 weeks (ongoing)
Freezing Trials – Room Temp

Freezing method

None (control)

-20°C (-4°F)

-80°C (-112°F)

Number of cells plated

1:5 serial dilutions, 4 μl per spot, strain used is Wyeast 1056

- After freezing treatment, cells stored at -20°C (-4°F)
- Original concentration is ~74M CFU/ml
- Survival of cells is less than 20% via this treatment
- Slightly better in -20°C
Freezing Trials – Chilled First

Freezing method

- None (control)
- -20°C (-4°F)
- -80°C (-112°F)
- Dry Ice (-109°F)
- EtOH Bath (-109°F)

Number of cells plated

1:5 serial dilutions, 4 μl per spot, strain used is Wyeast 1056
Freezing Trials – Chilled 3 hours

• After freezing treatment, cells stored at -20°C (-4°F)
• Original concentration is ~76M CFU/ml
• Survival of cells ~3% on dry ice or in an ethanol bath
• Survival is ~22% when frozen at -80°C
• When frozen at -20°C directly, survival is ~37%
Freezing Trials – Which is best?

• When freezing at -20°C, pre-chilling will more than double survival (36.6% vs. 16.5%)

• Affecting factors
  – Rate of cooling
  – Presence of cryoprotectants
  – Length of time stored at 4°C prior to freezing
Storage Trials

• Trial 1
  – Stored unprotected in freezer
  – NO viability after 16 weeks
Storage Trials

• Trial 1
  – Stored unprotected in freezer
  – NO viability after 16 weeks

• Trial 2
  – Why power is important
Storage Trials

• Trial 1
  – Stored unprotected in freezer
  – NO viability after 16 weeks

• Trial 2
  – Why power is important

• Trial 3
  – Why locks are good to have
Storage Trials – Number 4

• Room temp and refrigerated have highest viability after 4 weeks
• Samples lacking glycerol are dead
• Small difference between samples stored in cooler vs. not
• Difference between samples in cooler submerged in isopropanol or not
Other resources

- Zymurgy March/April 2007, Maribeth Raines
- First Steps in Yeast Culture (Pierre Rajotte)
References

- Park, J., Grant, C.M., Atfield, P.V., and I.W. Dawes. The freeze-thaw stress response of the yeast Saccharomyces cerevisiae is growth phase specific and is controlled by nutritional state via the RAS0-cyclic AMP signal transduction pathway. 1997 Applied and Environmental Microbiology (63) 3818-24.
Thanks for listening!

Feel free to email me at: tschmidlin@earthlink.net

This presentation and updates should (soon) be available at: cascadebrewersclub.org/knowledge/yeast/