Comparison of Media for Intermediate-term Storage of Brewer's Yeast

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Background

The number of microbreweries has been increasing in Minnesota over the past decade. To make these businesses economically viable, many microbrewers have begun to maintain their own stocks of yeasts. To create a success in beer production long-term storage of brewer's yeast is significant. These forms of storages have an effect on the quality and turn-around of the resulting beer, for it protects and preserves the yeasts' physiological properties. One method known for the success of long-term yeast storage is through cryopreservation, in which yeasts are frozen in an appropriate storage medium. Ideally for best results, brewers should conduct freezing in liquid nitrogen for all vegetative cells will remain at complete inactivity. However, it can be questioned if brewer's yeast can be stored intermediately through refrigeration as it is a simple an inexpensive way to create results and maintain viability. There was no clarification regarding different methods of cryopreservation, nor was there an establishment of which media are appropriate for yeast storage, and if that storage condition would be appropriate for all strains.

Method

- Two different strains of yeast (ale yeast and lager yeast) were obtained from a microbrewer and grown in wort broth in preparation for cryopreservation.
- Equal numbers of each strain were frozen in single use aliquots in wort broth freezing medium (WB, wort broth with 10% glycerol), CryoBroth (CB, Oxyrase, Mansfield, OH) and OxyStasis broth (OS, Oxyrase) . WB and CB aliquots were stored at -30^{o}C, and OS aliquots were stored at -20^{o}C in a frost-free freezer for up to 3 months.
- Cell densities and viability were assessed using ViaCount reagent (Millipore, MA), which stains viable cells green and dead cells red, followed by flow cytometry (Millipore).

Results

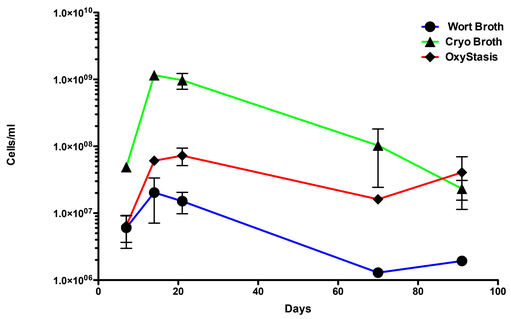
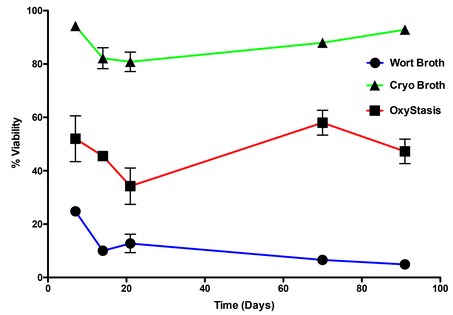


Figure 1: Viable Cell counts of ale yeast thawed from three different freezing media. After thawing, cells were washed twice in PBS and stained with Via-Count reagent.

Figure 2: Percent of viable ale yeast cells present in freezing medium. The number of cells staining green with ViaCount was divided by the sum of green staining and red staining yeast cells.



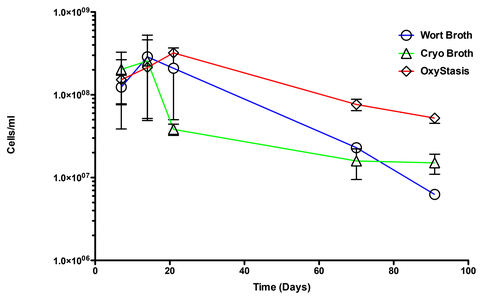
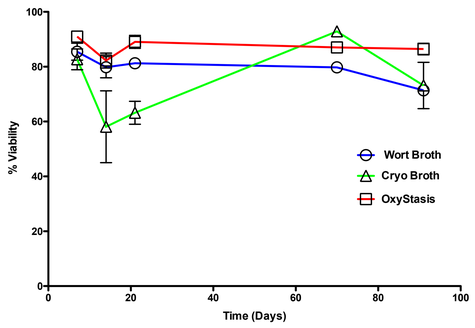


Figure 3: Viable Cell counts of lager yeast thawed from three different freezing media. After thawing, cells were washed twice in PBS and stained with ViaCount reagent.

Figure 4: Percent of viable lager yeast cells present in freezing medium. The number of cells staining green with ViaCount was divided by the sum of green staining and red staining yeast cells.



Conclusions

- Overall, ale yeast was preserved best in CB. Although the number of viable yeast cells in CB was similar to that observed in OS (Fig. 1), the percent viability of this strain was considerably higher in CB than in OS throughout the study. The viability of ale yeast in WB was comparatively poor.
- Lager yeast was preserved well in all three media, athough OS yielded higher cell counts than yeasts preserved in the other two media. The percentage of viable cells obtained from each medium was was similar.
- Our results indicate that no one medium is best for intermediate-term storage, and the medium most appropriate for storage should be determined experimentally for each strain.
- ViaCount reagent is lethal for yeasts. In addition to freezer damage, the length of time needed for each measurement may influence viability. Care should be exercised in experimental design so that fewer cultures are analyzed per batch.
- Cryopreservation in WB and CB require storage at temperatures -30^{o}C and below to prevent cell damage and subsequent death due to freeze-thaw cycles. However, OS does not freeze, allowing strains to be stored in household frost-free freezers. This is an attractive option as a cost containment measure, especially for microbrewery start-ups.

Acknowledgements

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References

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