The Development of a Density Based Screen to Identify Yeast with Higher Lipid Content

Kate Lansen1, Montie Vanden-Nest2, Basil Nikolau1
1 Center for Biorenewable Chemicals (CBiRC), Iowa State University, Ames, IA 50011
2 Des Moines Public Schools, Des Moines IA 50316

Abstract

The goal of this project is to create a new density-based centrifugation protocol to screen for yeast cells that have a high lipid content and, possibly, high fatty acid production. This test is based on the fact that cells with more lipids will be less dense and should, therefore, migrate higher in a centrifugation-generated density gradient. Prior to optimizing centrifugation conditions, we synchronized the cells to a single stage in the cell cycle using a Ch frameborder="0" src="https://example.com" class="image" height="400" width="400" />

Figure 1.  dSynchronization Experiments

Wildtype  
gl3, gl4

Not an Factor

Methods

Identification of yeast cells with lipid accumulation

Optimization of cell synchronization of wildtype and gln3, gln4 strains

Test density gradient centrifugation

Results & Discussion

The goal of this research is to develop a screen for the identification of yeast with high lipid content. To develop the method, we use a high lipid yeast strain (gln3, gln4) which lacks lipases and accumulates lipids) and a wildtype strain. Cells with higher lipid content (e.g., gln3, gln4) should have lower buoyant densities than cells with normal lipid content and should, therefore, separate over a density gradient. However, other factors, such as stage in the cell cycle, could also affect the buoyant density of cells. Therefore, we wanted to first synchronize our cells to a single stage using the mating phenotype, Δ-factor. Δ-factor is secreted by yeast cells of mating type a to attract cells of the opposite mating type. This phenomenon induces type a cells to adopt shmoo morphology (Figure 2) and arrests them at the G1 phase of the cell cycle. To optimize Δ-factor synchronization, 1 x 10^8 yeast cells were treated with different concentrations (0 µM, 30 µM, 50 µM, and 100 µM) for different lengths of time (50 min, 120 min, 150 min and 180 min) (Figure 3). Figure 3 shows that 95% of cells treated with 50 and 100 µM Δ-factor were synchronized and had formed shmoo at the 120-180 min time points.

Upon optimizing Δ-factor synchronization, we tested synchronized and asynchronous wildtype and gln3, gln4 cell strains for differences in cell density using density-based centrifugation. Gln3 cells with increased lipid content (in this case, gln3, gln4) should float higher in a density gradient than wildtype. Cells either treated (synchronized) or not treated (asynchronous) with Δ-factor were layered on top of different concentrations of Percoll, a medium that forms a density gradient during centrifugation, and centrifuged at different speeds and lengths of time. In addition, we tested whether cells needed to “recover” from the Δ-factor treatment by washing the cells and then growing them for one cell cycle prior to density gradient centrifugation.

The results suggest that Δ-factor synchronization does not necessarily improve the separation of the gln3, gln4 lipid accumulating mutant from wildtype because synchronized vs. asynchronous cells of the same genotype do not float at a different density. However, the gln3, gln4 mutant does migrate to a higher position than wildtype. This observation is consistent with our expectation that this lipid-accumulating mutant from wildtype because synchronized vs. asynchronous cells of the same genotype migrate to different positions in the Percoll gradient. The difference in density between gln3, gln4 and wildtype is not as large as expected. This could be due to the fact that yeast cells in this experiment were in early log phase and, in such a phase, the ideal (ideal) for synchronization, whose fatty acid accumulation has been shown to be highest during the stationary phase of yeast growth. This study demonstrates that 1) the stage of the cell cycle does not seem to greatly affect buoyant density of the cells and 2) that yeast cells of varying lipid contents can be separated based on buoyant density. In the future, we will perform density-based centrifugation on cells in stationary phase to hopefully improve the separation of wildtype from mutant cells that accumulate larger amounts of lipids. Although the method is not totally optimized, the current data suggests that a density-based screen for yeast strains with increased lipid content is feasible.

REFERENCES


ACKNOWLEDGEMENTS

The NSF Engineering Research Center for Biorenewable Chemicals. This material is based upon work supported by the National Science Foundation under Grant No. EEC-0813570.

Dr. Addis Lehman-Adams and RIT program for providing the internship opportunity.

Dr. Russ Nelms for opening his lab to this internship program.

Dr.RESULTS...