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High-Throughput Disruption of Yeast in a 96-Well Format

SUBJECT (SP018): High-throughput disruption of yeast
APPARATUS: Geno/Grinder®
APPLICATION: DNA/RNA and Other Extractions

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The wealth of information generated from years of biochemical, genetic, and molecular analyses has made yeasts both model biological systems and tools for bio pharmaceutical scientists. Consequently, yeast are a popular host for gene expression studies and for the production of recombinant proteins. Though many yeast species are in use, including Pichia, Hansenula, and Debaryomyces, the most popular yeast continues to be Saccharomyces. Yeast mRNA and intracellular proteins are often times difficult to extract intact from cells by traditional enzymatic methods. Lysing enzymes are often crude preparations containing RNase and proteases that will not only attack the cell wall, but also the molecules of interest. Furthermore, protoplasts generated from enzymatic digestion usually require lysis with detergents that will also denature many proteins to inactivity. Therefore, mechanical cracking/fracturing of the yeast cell is often required to liberate the molecules.

Mechanical disruption of yeasts has traditionally been accomplished by using either a press or bead mill (i.e., bead beater). In both approaches, samples are processed individually. For experiments where large numbers of yeast clones must be examined in a high-throughput screening environment, individual processing is a major bottleneck and impractical. Consequently, a method is needed that combines mechanical disruption of cells in a high-throughput format. The Geno/Grinder, a bead mill originally designed to smash seeds in deep well plates, can be used to disrupt yeast in a microwell plate format.

Materials and Methods

Saccharomyces cerevisiae was cultured overnight in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30 °C with agitation (150 rpm). Using a sterile 96-well microtiter plate (Corning Life Science Products), 100 µL of culture broth was added to each well along with 50 mg of acid washed glass beads (Sigma, G-8772). A range of glass bead sizes was used including 106 mesh (very small), 150–212 mesh, 212–300 mesh, and 425–600 mesh (large). Culture broth without glass beads was also prepared as a negative control. The plate was sealed with a rubber-sealing mat and locked into the Geno/Grinder. The yeast cells were disrupted for 5 and 10 minutes at 1500 rpm. Following disruption, the yeast cultures were examined by phase contrast microscopy and photographed.

Results and Discussion

The efficiency of yeast disruption is dependent upon the size of the glass beads and the duration of the grinding. Figures 1-3 demonstrate the efficiency of cell disruption with 425–600 mesh glass beads at 5 and 10 minutes. Lower mesh glass beads were less effective in cracking cells with the 106 mesh beads being particularly ineffective. It is concluded that 425–600 mesh glass beads are most suited for disrupting Saccharomyces. The design of the Geno/Grinder is also believed to be a factor in the disruption process. Standard bead mills adapted to microwell plates are modeled after “paint shakers” and move the plates in a “figure-eight” motion. This motion is not believed to lead to uniform cell disruption. The Geno/Grinder is designed to effectively disrupt cellular materials by oscillating the plate vertically. This motion allows glass beads to impact the cells more directly than standard mills where beads impact the well walls in addition to the cells.

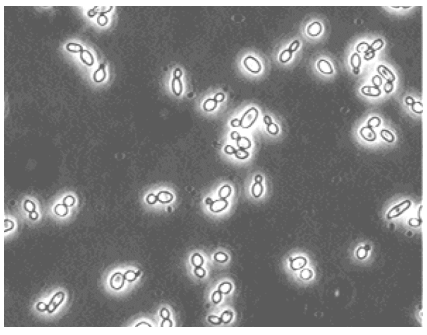

Figure 1. Negative control yeast without glass beads, the yeast remained intact after processing for 10 min. in the Geno/Grinder.

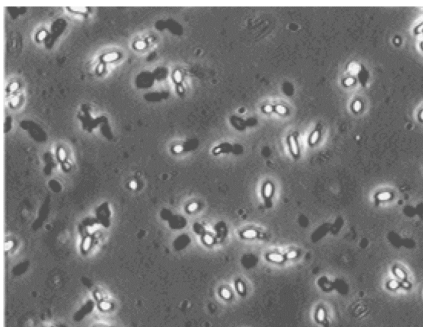

Figure 2. Yeast disrupted for 5 min. with 425–600 mesh glass beads, cracked yeast appear as dark “ghosts” while intact yeast continue to refract light and appear as bright.

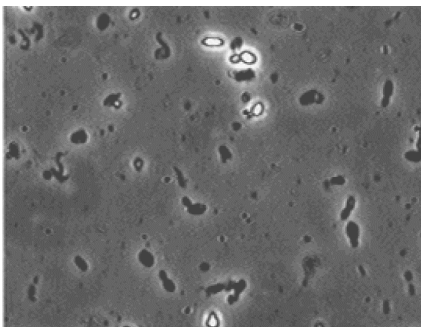

Figure 3. Yeast disrupted for 10 min, with 425–600 mesh glass beads, most yeast have been effectively disrupted as is demonstrated by cell ghosts and cellular debris. Some intact yeast remain.

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