Cramer, C. L., J. L. Ristow and R. H. Davis

New Instrument for rapid cell breakage.

A new instrument for disruption of microbial cells, the "Bead-Beater', has recently become available. We find it extremely rapid, thorough, and inexpensive in our work on organelle isolation. It is preferable to the mortar-and-sand or glusulase-digestion methods we have used in the past.

The Bead-Beater has 20-, 60-, and 340-ml chambers, suitable for a large range of sample sizes with comparable breakage kinetics. When using the 340-ml chamber, for example, the chamber is half-filled with buffer-wetted glass beads (300-500 µm diam.). A moist mycelial pad of l-4 g (dry-weight equivalent) is added together with buffer to fill the chamber completely. The Teflon impeller (rotor) is screwed on and the assembly is set on the blender motor. A simple and effective ice jacket (optional) is available. Blending for 30-sec pulses 30 sec apart assures adequate cooling. Almost complete breakage takes place in 1 min for 2.5 g of material; 4-5 min are needed for 10 g. The beads are removed from the homogenate and rinsed by filtering through cheesecloth. (The beads may be reused after detergent and acid washing.) The homogenate is then processed by differential centrifugation for organelles and/or soluble fractions. Our procedures involve an initial 5-min centrifugation at 600 x g, and filtration of the supernatant through glass fiber filters (934-AH, Whatman, Ltd.). The filtration step effectively removes fine cell-wall fragments without loss of organelles. The filtrate is centrifuged at 1O 15,000 x g for 20 min to obtain the crude organellar pellet. This supernatant liquid can then be centrifuged at 40,000 x g for 40 min to obtain a fraction enriched in plasma membranes. Re-extraction (3x) of the 600 x g pellet increases total yields of plasma membrane. (B.J. Bowman, personal communication.)]. Organelles (mitochondria and vacuoles) are similar in their physical end functional characteristics to those isolated by the best of the other methods (Davis et al. 1980 J. Bacteriol. 141: 144; Lambowitz et al. 1972 J. Biol. Chem. 247: 1536; Vaughn and Davis 1981 Molec. Cell Biol. 1: 797; Weiss et al. 1970 Eur. J. Biochem. 14: 75). A further description of the Bead-Beater procedure and the rapid isolation of pure mitochondria and vacuoles will be published elsewhere.

The advantage of the Bead-Beater is the thoroughness and spped of cell breakage. The glusulase method is somewhat more gentle, giving a greater proportion of intact organelles per broken cell. However, the increased efficiency of breakage with the Bead-Beater results in equivalent yields of organelles per gram of material. Moreover, the Bead-Beater method avoids both the long incubation and washing periods, and the danger of contaminating organelles with hydrolytic enzymes from the glusulase preparation. In the organellar pellet (15,000 x g) from wild type grown on minimal medium, we routinely get 50% of mitochondria and 25% of vacuoles in the intact state. It should be noted that the kinetics of breakage vary somewhat with the amount of material, the osmoticum, the strain, and the nutritional status. The major consideration in determining the time of disruption is to optimize breakage of cells, but to minimize the exposure of organelles to shearing after their release from cells. Thus, the shorter the time needed to disrupt cells, the fewer organelles

will themselves be disrupted. Because large batches require longer homogenization for comparable breakage, we recommend processing successive, smaller lots of mycelium for organelle recovery.

The Bead-Beater may be used for whole-cell extracts if large amounts of mycelia need to be homogenized extensively. These preparations may be somewhat dilute: bead-free, broken cell extracts have an approximate volume of 15-, 40-, and 250-ml, from the three chambers, respectively. However, ammonium sulfate precipitation can be applied to the extract, allowing soluble proteins to be concentrated by centrifugation.

The Bead-Beater is available from Biospec Products, P.O. Box 722, Bartlesville, OK 74003 for about $400. The company also sells the glass beads. It is also available from Tekmar, P.O. Box 37202, Cincinnati, OH 45222, (800)543-4461. Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA 92717.