Experience with the Applegate-Nelson-Metzenberg method of mutant enrichment in high sorbose medium

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Abstract
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The experiments reported here utilized 15% -25% continuous linear sucrose (Beckman, ribonuclease-free) gradients made in 50 mM Tris-HCl, pH 7.8 buffer containing 500 mM KCl, 5 mM MgCl₂ and 1 mM dithiothreitol (DTT). The gradients were refrigerated for 60 minutes at 10°C to stabilize them prior to centrifugation. The sample, ribosomes isolated from wild type Neurospora crossa and stored at -70°C (S.C. Schlitt and P.J. Russell 1974 J. Bacteriol. 120: 666-671), was thawed and immediately layered upon the gradient. Gradients were centrifuged in the TV850 rotor for 95 minutes, 47,000 rpm at 4°C. After centrifugation, gradients were displaced upwards through a flow cell to monitor nucleic acid absorbancy at 260 nm. This method of separation results in significantly greater resolution of the 60S and 37S ribosomal subunits (Fig. 1A) when compared with that achieved with the usual separation technique in which gradients are centrifuged in a Beckman SW27.1 swinging bucket rotor for 21 hours, 24,000 rpm at 4°C (Fig. 1B). Another advantage of this technique is that it overcomes the frustrating problem of partial degradation of the 37S subunit during centrifugation of gradients in the SW27.1 rotor. Quantification of this 37S degradation, using 60S:37S peak amplitude ratios (PAR) indicates that TV850 gradients result in a mean PAR of 1.5:1 and a substantially increased resolution of the 60S and 37S subunits when compared to SW27.1 gradients which result in a mean PAR of 3:1 for the two subunits.

![Relative position in gradient](image)

Figure 1. -- Zone sedimentation profiles of wild type ribosomal subunits produced by various means: A. Centrifugation in TV850 vertical rotor, 95 min, 47,000 rpm; B. Centrifugation in SW27.1 swinging bucket rotor, 21 hr, 24,000 rpm; C. Maintenance of ribosome sample at 0°C for 3 hr, then centrifugation in TV850 rotor as in A.

Having established an optimal method for ribosomal subunit separation and analysis, we examined the parameters for the preparation and storage of ribosomes prior to gradient analysis. We have observed a correlation between the duration of handling ribosomes prior to layering on sucrose gradients and successful subunit separation. This led us to investigate the effects of temperature on subunit stability. After thawing, ribosomes were stored in an ice bath for 3 hours prior to layering on top of a 15% - 25% continuous gradient and centrifuging in the TV850 rotor as already described. A highly aberrant profile (Fig. 1C) was obtained in comparison with the control (Fig. 1A). Repeated freeze-thawing of the sample also had detrimental effects on both 37S stability and subunit separation. In summary, ribosomal subunits are quickly and efficiently separated in a vertical rotor when layered on 15% - 25% sucrose gradients immediately after thawing. (Supported by Grant GM-22488 from N.I.H. and NSF Grant PCM76-21478.)

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The high-sorbose, filtration concentration method of mutant enrichment reported by Applegate et al. (Neurospora Newsl. 25: 17, 1978) was modified and evaluated for its efficiency of mutant selection. Conidia were suspended in water, filtered through four layers of cheesecloth (20 mesh/inch), adjusted to 2 x 10⁶ - 2 x 10⁷/ml, and 10 ml of the suspension was placed in a 10 cm diameter glass petri dish for one min exposure to W-light (48 ergs/sec/mm²) that resulted in 70 to 90%
METHODS FOR STOCK PRESERVATION

Catcheside, D.E.A. 1 and D.G. Catcheside 2.

Stocks in the DGC collection are prefixed F, those in the DEAC collection are prefixed T.

We have each established extensive culture collections maintained on silica gel. Recently it has become apparent that while one of the collections has maintained full viability, the other has not. Comparison of the methods for preparation and conditions of storage suggest that two factors may be involved in determining this differential viability: the number of conidia per gram of gel and the control of moisture regain.

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The materials and methods used are similar to those described by D.D. Perkins (1977 Neurospora News 24: 16-17). For the T stocks, cultures were grown on 1.5 ml slopes for 5-7 days and conidia were suspended in 1.5 ml water and then mixed with 1.5 ml of reconstituted nonfat milk (10 g powder/100 ml, autoclaved 5 min at 10 psi and steamed for 30 mins on two successive days). Conidia were allowed to settle and half or more of the supernatant was discarded. The conidia were resuspended in the remaining supernatant and up to 0.8 ml was distributed evenly onto about 3.59 of silica gel (12-20 mesh dry sterilized at 180°C for 1.5 hrs in 3x100 mm tubes closed with plugs rolled from cheese cloth; the tubes were stored over anhydrous CaCl2 prior to use). The tubes were tapped or vibrated mechanically to distribute the inoculum evenly but were not cooled during gel hydration. The gel became dry and free running within a few minutes. Tuber were kept in vacuo over anhydrous CaCl2 for 3-5 days prior to checking viability and closing with Parafilm. The sealed tuber have been kept at 4°C in heavy duty plastic bags containing open tuber of indicator gel which are replenished as required. Parafilm closures have needed replacement at about 5 year intervals. For the F stocks, the only identifiable variations from the methods used for the T stocks were: i) the conidial concentrations step was omitted and ii) following closure, the tubes were stored in plastic bags which did not contain desiccant.