LARGE-SCALE PREPARATION OF YEAST "SOLUBLE" RIBONUCLEIC ACID

Robert W. Holley

U. S. Plant, Soil and Nutrition Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Department of Biochemistry, Cornell University, Ithaca, N. Y.

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Preparation of large amounts of "soluble" RNA is essential for structural studies of amino acid-specific "soluble" RNAs. In order to simplify large-scale preparations, the procedure for preparation of yeast "soluble" RNA (Holley, Apgar, Doctor, Farrow, Marini and Merrill, 1961) was reexamined, and the procedure has been modified to simplify handling large quantities of yeast. The major modifications are, (1) the phenol, water and yeast are mixed by stirring in a large polyethylene tank rather than by shaking in glass-stoppered bottles, and (2) the phenol and aqueous phases of the mixture are allowed to separate by gravity, thus avoiding the centrifugation of large volumes of phenol-water-yeast mixture. The modified procedure, described below, is applicable to 100 lb. of yeast and yields approximately 65 g of "soluble" RNA. The RNA obtained is indistinguishable from that isolated by the earlier procedure. It is of interest that there is no inactivation of the RNA, under the conditions of the preparation, during nine days at room temperature.

EXPERIMENTAL

The entire procedure was carried out at room temperature.

One hundred pounds of fresh, pressed cakes of Bakers' yeast (Fleischmann's)* were crumbled into a 55-gallon (200-l) polyethylene tank with

*Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment of the product listed by the U. S. Department of Agriculture.

spigot. (Polyethylene gloves were worn to avoid contamination of the yeast with nucleases (Holley, Apgar and Merrill, 1961).) To the yeast was added 42.7 l (the contents of eighteen 5-pint bottles) of 88% phenol* (Mallinckrodt, analytical reagent, liquefied, for chromatography), followed by 100 l of demineralized water. The mixture was stirred until the yeast was mixed thoroughly, and stirring was repeated occasionally for 1 hr. The tank was covered, and the phenol and aqueous phases were allowed to separate by gravity.

After one week, approximately 29 cm (65 l) of the clear upper (aqueous) layer were siphoned into a second 55-gallon polyethylene tank and mixed with 3.5 l more phenol. The phenol was allowed to separate overnight and was removed from the bottom of the tank by a tube connected to a suction bottle. The aqueous solution in the 55-gallon tank was thoroughly mixed with 600 ml of 20% potassium acetate, pH 5.2, and 125 l (35 gallons) of 95% ethanol. The following day the clear aqueous-alcohol supernatant was siphoned off and discarded. The crude RNA was isolated from the remaining slurry by centrifugation in wide-mouth 32-ounce polyethylene bottles in an International size 2 centrifuge. The precipitate was washed, by resuspension and recentrifugation, once with 95% ethanol and once with ether.

Nine days from the start of the preparation, as much as possible of the remaining clear aqueous layer (approximately 9 cm, 20 l) was siphoned from the phenol-yeast mixture**. It was mixed with another liter of phenol, the phenol extract was discarded, and the RNA was precipitated by the addition of 200 ml of potassium acetate, pH 5.2, and 40 l of 95% ethanol. The crude RNA was isolated and washed as above.

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*Phenol causes serious burns and must be handled with great care.

**Care must be exercised in the disposal or recovery of the phenol. In the preparation described, the phenol-yeast mixture was allowed to flow from the spigot of the tank into 5-gallon cans, and the mixture was removed and burned by the Cornell University Safety Division, to whom we are indebted.

The combined crude "soluble" RNA was purified on a column of 750 g of DEAE-cellulose (Selectacel 70, Standard) prepared in a polyethylene cylinder 15.5 cm in diameter by 45 cm high (Nalgene pipet jar size D). The bottom of the cylinder was fitted with a short piece of polyethylene tubing threaded to fit a hole drilled and threaded in the bottom of the cylinder. A 5-cm-thick pad of glass wool was placed in the bottom of the cylinder and 750 g of DEAE-cellulose, previously washed by decantation with 0.1 M Tris-chloride buffer, pH 7.5, was poured into the column as a slurry in the buffer. The crude RNA was dissolved in 10 l of 0.1 M Tris-chloride buffer, pH 7.5. The small amount of insoluble material was allowed to settle and was finally separated from the last of the RNA solution by centrifugation. The solution was allowed to flow into the DEAE-cellulose column at the unrestricted flow rate of the column, and the column was then washed with 60 l of the 0.1 M Tris buffer. The RNA was eluted from the column with 20 l of 1 M sodium chloride in 0.1 M Tris-chloride buffer, pH 7.5. The eluate was mixed with 40 l of 95% ethanol. The next day the clear supernatant was siphoned off and discarded. The RNA was isolated from the remaining slurry by centrifugation in wide-mouth 32-ounce polyethylene bottles. The RNA was washed once with 2 l of 80% ethanol and twice with 2 l of 95% ethanol, and was dried in a vacuum desiccator over phosphorus pentoxide. The yield was over 65 g.

References

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