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Extraction of RNA from Ca-Alginate Encapsulated Yeast for Transcriptional Profiling

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Abstract

We have developed a method for preparing high-quality total RNA from Ca-alginate encapsulated *Saccharomyces cerevisiae* that is suitable for microarray analysis. Encapsulated cells were harvested from immobilized cell reactors and flash-frozen in liquid nitrogen. Following low-temperature mechanical disruption, cells were freed from Ca-alginate by reverse ionotropic gelation, purified by centrifugation, and total RNA was extracted using hot acid phenol. The yield and quality of the RNA were consistently high; the RNA was free of contaminating alginate, and in microarray analysis performed as well as RNA isolated from planktonic cells.

Keywords

Alginate-encapsulated yeasts; RNA isolation; Expression profiling

NOTE

DNA microarrays make it possible to interrogate the cellular transcriptome in response to diverse experimental challenges [1, 2]. Systematic compilation of global expression profiles over the last decade has illuminated the structure and regulation of metabolic networks, and accelerated the pace of genome annotation in many species, notably bakers' yeast, *Saccharomyces cerevisiae* [3-7]. Our group is studying metabolic regulation in yeast cells encapsulated within Ca-alginate beads. Encapsulated yeast achieves rates of ethanologenic fermentation many-fold higher than continuously-fed planktonic (free-floating) cells of the same genotype, owing to greatly diminished flux to biomass [8]. Our experiments require direct and reliable comparisons between planktonic yeast grown in batch or chemostat

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cultures and alginate-encapsulated cells packed into continuously-fed immobilized cell reactors (ICR). As expression profiling is an essential element of our research program we needed to isolate high-quality RNA from immobilized yeast. Although alginate-encapsulated microbial cells have been studied for over two decades [9,10] purification of RNA from such cells has not been previously reported. The most commonly used protocol for extracting high-quality RNA from yeast is based on the hot acid phenol method first described by Schmitt et al. [11]. However, RNA and alginate co-purify using this method, and alginate absorbs strongly at 260 nm (data not shown). We therefore developed a protocol that separates intact yeast cells from their alginate matrix under conditions that suppress RNA degradation, followed by RNA extraction from the isolated cells.

S. cerevisiae strain S288c (*MAT α SUC2 gal2 mal mel flo1 flo8-1 hap1*) used in this study was obtained from the American Type Culture Collection (ATCC No. 204508). Planktonic cell culture studies were performed essentially as described (12). Preparation of immobilized beads was carried out as described earlier (10). Basically, cells were encapsulated by mixing them with an aqueous solution of alginic acid, and adding this mixture dropwise to a 0.2 M CaCl_2 solution. Ca^{++} cross-links alginic acid monomers, and yeast cells are immobilized in the resulting porous polymeric matrix.

Alginate-encapsulated yeast in 3mm diameter beads was cultivated in ICRs as previously described (13). Samples were periodically withdrawn, frozen in liquid N_2 , and stored at -80°C . Following encapsulation, cells soon cease to divide; after 72 h the average cell number per bead is constant at approximately 2×10^7 cells. Approximately twenty frozen beads (equivalent to ~ 1 ml) containing ca. 4×10^8 cells were ground to a coarse powder in a mortar and pestle in the presence of liquid N_2 . The powder was transferred to a 50 ml conical tube and suspended in 5 ml ice-cold 0.2 M EDTA (pH 8.0) by vortexing. Five ml of ice-cold RNA-Later (Ambion, Austin TX) was added, and the mixture was vortexed vigorously for 30 sec. The volume was brought to 50 ml with ice-cold RNase-free sterile water. Thereafter, the suspension was held on ice for 30 min and vortexed intermittently, during which time the Ca-alginate matrix dissolved. Particulate debris from the beads was removed by sieving the suspension through a strainer (mesh size approx. 1 mm) into a fresh conical tube. Yeast cells were recovered by centrifugation at $1118 \times g$ for 5 min in a swinging bucket rotor at 4°C . The pellet was resuspended in 5 ml of 0.2 M EDTA, 5 ml of RNA-Later and 40 ml of RNase-free H_2O and centrifuged as above. The resulting pellet was resuspended in 50 ml of ice-cold RNase-free H_2O and centrifuged again. The cell pellet was resuspended in 0.4 ml AE buffer (50 mM Na-acetate, 10 mM EDTA, pH 5.0), and transferred to a screw-cap microcentrifuge tube. 40 μl of 10% SDS was added and the contents were mixed. Then 0.5 ml acid phenol-chloroform (Ambion, Austin TX; preheated to 65°C) was added and mixed. The tube was incubated at 65°C for 15 min with periodic mixing and then centrifuged briefly. The aqueous phase was transferred to a fresh tube and extracted again with hot acid phenol-chloroform; the resulting aqueous phase was then extracted with chloroform- isoamyl alcohol (24:1) (Ambion, Austin TX). Finally, the aqueous phase was brought to a volume of 0.4 ml with AE buffer to which 40 μl of 3M Na-acetate (pH 5.0) was added. RNA was precipitated by adding 1 ml of 95% ethanol. Following overnight incubation at -20°C , RNA was pelleted by centrifugation at $13000 \times g$

for 20 min in a microcentrifuge; the pellet was washed with 70% ethanol, dried, and resuspended in 200 μ l RNase-free H₂O (Ambion, Austin TX).

Planktonic batch-cultured yeast were harvested and processed for RNA essentially as described [14]. Total RNA from planktonic and immobilized yeast was analyzed spectrophotometrically using a BioMate uv/vis spectrophotometer (Thermo-Scientific, Waltham, MA). Analysis of RNA size and integrity was performed on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA) using the RNA 6000 Nano-chip kit according to the manufacturer's instructions.

To test the efficacy of our protocol, we extracted RNA from immobilized *S. cerevisiae* and compared it to RNA extracted from planktonic cells. The RNA extracted from three biological replicates of planktonic cells from day 5 of batch cultivation had mean absorbance ratios at 260 nm and 280 nm (260/280) of 1.91 ± 0.17 . The 260/280 of RNA extracted from three samples of encapsulated yeast cells from day 5 of ICR fermentation was 1.85 ± 0.39 . This indicates that RNA extracted from both cell types was of comparably high purity. RNA yield (Mean $\pm$ S.D.) from planktonic cells was $86.7 \pm 22.3 \mu\text{g}$ (10^7 cells)⁻¹ while yield from encapsulated cells was $52.0 \pm 2.7 \mu\text{g}$ (10^7 cells)⁻¹. RNA yield obtained with our protocol is within the expected range of 30-135 μg per 10^7 cells [11].

Fig. 1 shows a representative BioAnalyzer profile of total RNA from day 5 biological replicates of planktonic and immobilized yeasts. The electropherogram shows that RNA samples extracted from planktonic yeast by the standard method and from immobilized yeast by the method described here are comparably intact, as evident from the sharp 18S and 28S rRNA peaks. The abundant low molecular weight RNAs correspond to 5S RNA and other intact low molecular weight species. The fact that smaller RNAs are not masked by breakdown products provides further evidence that the RNA is intact. Therefore we conclude that the RNA extracted from yeast encapsulated in Ca-alginate beads by the method described above is intact, pure, and suitable for mRNA analyses.

RNA samples obtained by this method yielded highly reproducible microarray results between samples taken at the same time-points from independent ICR fermentations (biological replicates). RNA isolated from immobilized cells by our protocol provided excellent material for microarray analysis using Affymetrix Yeast 2.0 GeneChips. Fig. 2A-D shows scatter plots of expression for RMA (Robust Multiarray Average) normalized values of two biological replicates from each of four timepoints (Day 1, Day 3, Day 5, and Day 10). Correlation coefficients for biological replicates for the four time points ranged from 0.85 to 0.98, with an overall average of 0.92 and standard error of 0.0085. Deviation from the diagonal identity line between independent observations is minimal, indicating that array results obtained using our RNA isolation procedure are highly reproducible.

These observations demonstrate that our protocol for extracting RNA from Ca-alginate-encapsulated yeast cells yields high-quality RNA, devoid of interfering alginate, that is suitable for expression profiling.

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Citations

1. Chambers D, Lumsden A. Profiling gene transcription in the developing embryo: microarray analysis on gene chips. *Methods. Mol. Biol.* 2008; 461:631–655. [PubMed: 19030827]
2. Steinberg CE, Stürzenbaum SR, Menzel R. Genes and environment - striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. *Sci. Total. Environ.* 2008; 400:142–161. [PubMed: 18817948]
3. Daran-Lapujade P, Daran JM, van Maris AJ, de Winde JH, Pronk JT. Chemostat-based micro-array analysis in baker's yeast. *Adv. Microb. Physiol.* 2009; 54:257–311. [PubMed: 18929070]
4. Bharucha N, Kumar A. Yeast genomics and drug target identification. *Comb Chem. High. Throughput. Screen.* 2007; 10:618–634. [PubMed: 18045076]
5. Sherlock, G.; Spellman, PT. Seven Years of Yeast Microarray Analysis. In: Dunn, MJ.; Jorde, LB.; Little, PFR.; Subramaniam, S., editors. *Encyclopedia of Genetics, Genomics, Proteomics and Bioinformatics*. John Wiley & Sons, Inc.; New Jersey: 2008. p. 1-9.
6. Zhou J. Microarrays for bacterial detection and microbial community analysis. *Curr. Opin. Microbiol.* 2003; 6:288–294. [PubMed: 12831906]
7. Conway T, Schoolnik GK. Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. *Mol. Microbiol.* 2003; 47:879–889. [PubMed: 12581346]
8. Doran PM, Bailey JE. Effects of immobilization on growth, fermentation properties, and macromolecular composition of *Saccharomyces cerevisiae* attached to gelatin. *Biotechnol. Bioeng.* 1986; 28:73–87. [PubMed: 18553844]
9. Galazzo JL, Bailey JE. Fermentation pathway kinetics and metabolic flux control in suspended and immobilized *Saccharomyces cerevisiae*. *Enzyme. Microb. Technol.* 1990; 12:162–172.
10. Hilge B, Rehm H. Comparison of fermentation properties and specific enzyme activities of free and calcium-alginate-entrapped *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 1990; 33:54–58.
11. Schmitt ME, Brown TA, Trumpower BL. A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. *Nucleic. Acids. Res.* 1990; 18:3091–3092. [PubMed: 2190191]
12. van Dijken JP, Bauer J, Brambilla JL, Duboc P, Francois JM, et al. An interlaboratory comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains. *Enzyme. Microb. Technol.* 2000; 26:706–714. [PubMed: 10862876]
13. Yadav BS, Rani U, Dhamija SS, Nigam P, Singh D. Process optimization for continuous ethanol fermentation by alginate-immobilized cells of *Saccharomyces cerevisiae* HAU-1. *J. Basic. Microbiol.* 1996; 36:205–210. [PubMed: 8676283]
14. Ferea TL, Botstein D, Brown PO, Rosenzweig RF. Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 1999; 96:9721–9726. [PubMed: 10449761]

Fig 1A-B. Electropherogram of total RNA from 5-day old cultures of planktonic or immobilized yeast

RNA from planktonic and immobilized yeast was isolated by the hot acid phenol method and by our protocol, respectively. Total RNA was analyzed on an Agilent 2100 BioAnalyzer using an RNA 6000 Nano-chip kit. A) The upper band in the BioAnalyzer scan image corresponds to 28S rRNA (~4000 nt), and the lower band (~2000 nt) corresponds to 18S rRNA. B) The X-axis in the electropherogram denotes molecular weight in nucleotides while the Y-axis denotes fluorescence intensity.

Fig2(A-D). Scatterplot comparisons of RMA (Robust Multiarray Average) normalized values from each of 2 biological replicates taken from immobilized cell reactors (ICR) on (A) Day 1, (B) Day 3, (C) Day 5, (D) Day 10

Transcript levels from immobilized cell cultures were analyzed by Affymetrix Yeast 2.0 GeneChips, using protocols developed by the manufacturer. A pair of observations of a single array feature is represented by a point on the scatter plot. The x—coordinate of the point corresponds to the measurement obtained from the first observation, and the y—coordinate, from the second. The tight clustering at the lower corner of the scatterplot is primarily due to very low signal intensities from hybridization to *S. pombe* probes on the Yeast 2.0 array.