Benchmarks

Extraction of nucleic acids from yeast cells and plant tissues using ethanol as medium for sample preservation and cell disruption

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BioTechniques 49:655-657 (September 2010) doi 10.2144/000113476
Keywords: cell wall disruption; cell wall dehydration; ethanol; zeolite; in situ preservation of RNA; small RNA

Supplementary material for this article is available at www.BioTechniques.com/article/113476.

Here we report that dehydrated ethanol is an excellent medium for both in situ preservation of nucleic acids and cell disruption of plant and yeast cells. Cell disruption was strongly facilitated by prior dehydration of the ethanol using dehydrated zeolite. Following removal of ethanol, nucleic acids were extracted from the homogenate pellet using denaturing buffers. The method provided DNA and RNA of high yield and integrity. Whereas cell wall disruption was essential for extraction of DNA and large RNA molecules, smaller molecules such as tRNAs could be selectively extracted from undisrupted, ethanol-treated yeast cells. Our results demonstrate the utility of absolute ethanol for sample fixation, cell membrane and cell wall disruption, as well as preservation of nucleic acids during sample storage.

Cell disruption in ethanol has long been used for the isolation of cell wall material from plants (1). Although not applicable to the isolation of native proteins or organelles, cell disruption in ethanol might be suitable for isolation of nucleic acids. Reports on long-term in situ preservation of DNA in ethanol are controversial. Whereas initial attempts to fix plant tissues in ethanol or other organic solvents showed rapid decay of DNA (2,3), 95% or 100% ethanol has more recently been successfully used for the in situ preservation of DNA in plant tissues (4,5). The aim of the current study was to reevaluate the suitability of ethanol as a preserving medium for RNA and DNA, especially during the mechanical disruption of biological samples with rigid cell walls, such as yeast and plant cells.

When yeast cells (complete materials and methods can be found in the Supplementary Materials) were ruptured in an aqueous buffer with zirconium oxide beads as previously described (6), complete cell disruption was not reached within 1 h. The mechanical force generated by vortex mixing with glass beads was insufficient to disrupt yeast cells in the solubilizing buffer or in 90% ethanol. However, in 96% ethanol, the majority of the cells were broken in ≤5 min when using the same homogenization procedure. Reduction of the water activity to near zero by means of zeolite beads markedly increased the homogenization efficiency (Figure 1, A–C).

When immersed overnight in absolute ethanol together with the zeolite beads, plant materials became brittle and could be completely disrupted with the zirconium oxide beads on the vortex shaker (Figure 1D). Comparable disruption required at least a 2-fold longer amount of time when performed without zeolite pretreatment (data not shown). The negative effect of a low water concentration is most likely the result of the cell wall polymers' high affinity for water. When water in the cell wall is completely removed, the scaffolds of fungal and plant cells walls become stiffer, and mechanical impulses are no longer efficiently damped by elastic deformation. While disruption with beads in ethanol is well-suited to microbial cells and thin plant structures, other techniques for fixation and disruption with ethanol might be necessary when working with bulkier plant tissues. Fixation and preservation in ethanol of

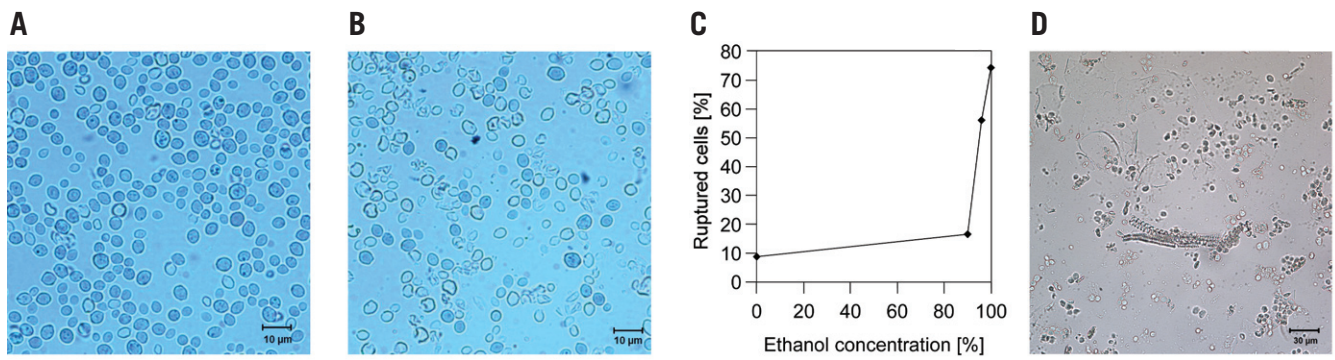

Figure 1. Disruption of biological samples in ethanol. Disruption was carried out with 8 mg (dry weight) yeast cells or 100 mg (fresh weight) leaf tissue in 1 mL buffer or ethanol. (A) Yeast cells equilibrated with 90% ethanol and then homogenized with glass beads on the vortex shaker for 5 min. (B) Yeast cells treated in the same way after prior dehydration in absolute ethanol with zeolite beads. Only intact cells accumulate methylene blue. (C) Efficiency of cell disruption following homogenization of yeast samples for 5 min with glass beads in solubilizing buffer (0%) and ethanol of different concentration. (D) Homogenized sample obtained from completely dehydrated rosette leaves of *Arabidopsis thaliana*. Scale bars, 10 µm in panels A and B and 30 µm in panel D.

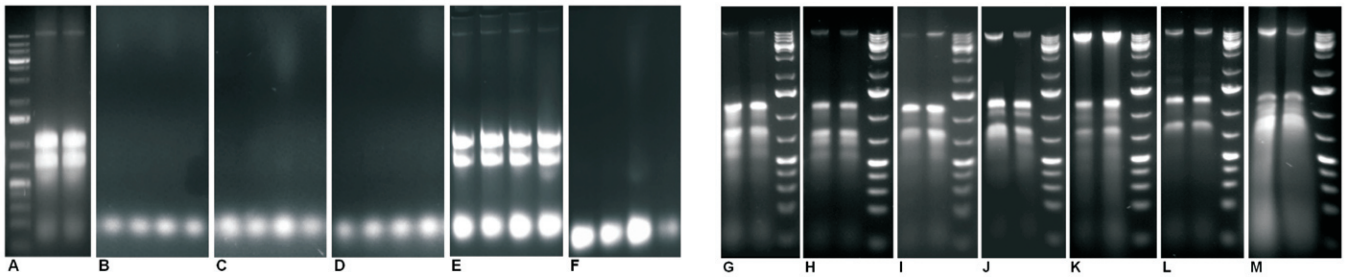

Figure 2. Ethidium bromide–stained agarose gels of nucleic acids extracted from homogenates and intact cells of *Saccharomyces cerevisiae* and homogenates of plant. (A–F) Yeast cells extracted after cell disruption by the standard protocol (A) and without the cell disruption step after preincubation in 50% ethanol (B), 70% ethanol (C), 96% ethanol (D), shaking in anhydrous ethanol with zeolite beads (E), and extraction on a glass filter with anhydrous ethanol (F). (G–M) Homogenates prepared from different plant tissues. Time of storage in ethanol is indicated in parentheses. (G) Siliques of *Arabidopsis thaliana* (1 day); (H) leaves of *A. thaliana* (3 days); (I) flowers of *A. thaliana* (30 days); (J) leaves of *Daucus carota* (30 days); (K) flowers of *D. carota* (60 days); (L) leaves of *Perlagonium zonale* (30 days); and (M) young needles of *Larix decidua* (2 days). Corresponding size markers (GeneRuler 1 kb Plus; Fermentas, St. Leon-Rot, Germany) are indicated. The multiple lanes in each gel correspond to extracts prepared in parallel.

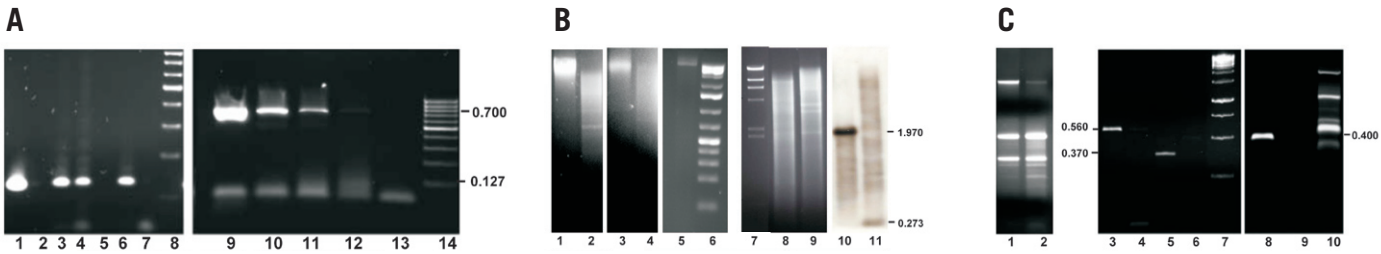

Figure 3. Applicability of DNA and RNA extracted after cell disruption in anhydrous ethanol. (A) PCR analysis of genomic DNA extracted from *Saccharomyces cerevisiae*. The gel on the left shows amplification of a 127-bp fragment of the 5.8 S rRNA. Lane 1, positive control of DNA isolated using DNeasy tissue kit (Qiagen, Hilden, Germany); lanes 2 and 5, undiluted raw extracts; lanes 3 and 4, DNA extracted according to the protocol with SDS removed by ethanol precipitation, template dilutions of 1:5 and 1:10, respectively; lane 6, DNA extracted according to the protocol with column-based purification instead of ethanol precipitation, template dilution of 1:5; lane 7, negative control without template. The gel on the right shows amplification of a 700-bp fragment of the actin (*act1*) gene using template DNA extracted according to the protocol, with SDS removed by column purification, and the DNA concentrated using ethanol precipitation. Lane 9, undiluted DNA; lanes 10–12, DNA diluted 10-, 100-, and 1000-fold, respectively; lane 13, negative control reaction without template; lanes 8 and 14, size markers (GeneRuler 100 bp; Fermentas). The sizes of the PCR fragments are marked (kb). (B) Restriction fragment analysis of DNA extracted from *S. cerevisiae*, *Arabidopsis thaliana*, *and Daucus carota*. DNA was extracted and column-purified by size exclusion chromatography with porous microcapsules (see the Supplementary Material and Methods). Lanes 1 and 2, leaves of *D. carota*; lanes 3 and 4, leaves of *Arabidopsis*. Digestion was carried out with *Eco*RI (lanes 2 and 4). Lanes 5 and 6, undigested DNA of *S. cerevisiae* and corresponding size marker (GeneRuler 1 kb Plus). Lanes 7–11, Southern hybridization of DNA isolated from *S. cerevisiae*; lane 7, size marker (λ-DNA/*Hin*dIII); lanes 8 and 9, total DNA was column-purified and digested with *Eco*RV and *Pvu*II, respectively; lanes 10 and 11, filter hybridization with a *S. cerevisiae* mitochondrial cytochrome c oxidase subunit II (*coxII*) gene DNA probe is shown. (C) Ethidium bromide–stained agarose gel analysis of RNA quality (lanes 1 and 2, denaturing gel) and RT-PCR (lanes 3–10). Lanes 1 and 2, NucleoSpin-RNA-cleanup (Macherey Nagel, Düren, Germany) column-purified extracts isolated from florets of *D. carota* and leaves of *A. thaliana*, respectively; lane 3, a 560-bp fragment of α-tubulin was amplified from cDNA generated from flowers of *D. carota*; lane 5, a 370-bp amplification product of the tubulin α-3/α-5 chain was obtained from cDNA of leaves of *A. thaliana*; lane 8, a 400-bp fragment of *act1* was amplified from cDNA of *S. cerevisiae*; lanes 4, 6, and 9, negative control reactions without reverse transcriptase; lanes 7 and 10, size markers (GeneRuler 1 kb Plus and GeneRuler 100 bp Plus; Fermentas, respectively), sizes of PCR fragments are indicated (kb).

thin leaves or small flowers is easy and safe. This is an important advantage relative to RNAlater (U.S. patent no. 6204375), since these plant materials tend to float upon that solution and be slowly immersed in it.

When yeast cells were disrupted in ethanol and extracted using the protocol described in the Supplementary Materials, the extracts contained high molecular weight DNA and high amounts of RNA (Figure 2A). In contrast, destruction of the lipid membranes by ethanol without cell disruption allowed for the subsequent extraction of small RNAs (Figure 2, B–D). Therefore, the sharp size permeation limit of the yeast cell wall (7–10) may be useful for selective extraction of small RNA molecules. It is noteworthy that yeast cell walls were partly disrupted when complete dehydration was carried out by slow shaking of the cells with the zeolite beads. In this case, rRNA and DNA appeared in the extracts (Figure 2E). Complete dehydration alone did not give this result (Figure 2F). For plant materials completely disrupted in ethanol, the extracts contained well-preserved RNA and DNA (Figure 2, G–M). After storage in ethanol for 1–2 months, high molecular weight nucleic acids remained extractable from mechanically disrupted yeast cells (data not shown) and plant tissues (Figure 2, I–L).

The yield of DNA extracted from yeast reached ~1%, and the yield of total nucleic acids was ~10% of dry weight (Supplementary Table S1). These yields are comparable to those of previous reports using dormant baker's yeast (11). An extraction time of 15 min was sufficient (Supplementary Table S2). Lower yields were obtained at an SDS concentration of 0.7%, with a 1.4% SDS concentration sufficient to obtain maximum yields if the ethanol was completely evaporated. Complete drying of the homogenate pellet from anhydrous ethanol was possible without loss of extractability. When homogenate pellets were dried completely from ethanol containing water, they were not easily dissolvable in the extraction buffers. From Supplementary Tables S1 and S2, it may be derived that storage of yeast cell homogenates for 1 month in absolute ethanol at room temperature does not affect yield.

RNA and DNA could be extracted and purified from the dried cell homogenate by standard protocols (see the Supplementary

Methods). After removal or dilution of SDS, the DNA could be used for PCR (Figure 3A). Digestion patterns obtained using *Eco*RI, *Eco*RV, or *Pvu*II (Figure 3B) demonstrate the suitability of column-purified DNA for applications requiring digested DNA samples (e.g., RFLP, AFLP analysis). Southern blot analysis of extracted yeast DNA using a 320-bp probe from the first exon of the mitochondrial gene cytochrome c oxidase II (*COXII*; V00685) is shown. Two distinct bands of size 1.97 and 0.27 kb can be seen, corresponding to the restriction fragment lengths deduced from the sequence of the mitochondrial genome. This provides further evidence of the DNA quality of the extracted samples.

RNA quality was visualized by agarose gel electrophoresis under denaturing conditions, showing a ratio of the intensities of 28S to 18S rRNA bands to be ~2 (Figure 3C, lanes 1 and 2). The quality of RNA in column-purified SDS extracts from *Saccharomyces cerevisiae* was high, as demonstrated by RNA integrity number (RIN) values of 9.0–9.2. Purification using acid-guanidinium thiocyanate-phenol-chloroform treatment typically resulted in RIN values of 8.0–8.8. Generally, RIN values of 8.0–9.2 reflect an RNA quality sufficient for downstream molecular applications. As shown for *Arabidopsis*, *Daucus*, and *Saccharomyces*, the purified RNA could be used for RT-PCR (Figure 3C, lanes 3–10; see the Supplementary Methods).

We have demonstrated that completely dehydrated cells and tissues are more easily disrupted in ethanol than hydrated ones, and the homogenates provided high yields of DNA and RNA with good structural integrity suitable for analyses using standard molecular biological methods. Furthermore, we showed the described method of cell disruption is compatible with established methods of RNA and DNA extraction and purification. The combination of efficient cell disruption with in situ preservation of RNA and DNA in ethanol offers an attractive alternative to both sample storage in the RNAlater solution and grinding with liquid nitrogen.

Acknowledgments

We thank Thomas John Buckhout for discussions and critical reading of the manuscript and Ekkehard Richter for support in microphotography. Financial support of this study by the program "Wirtschaft trifft Wissenschaft" of the Bundesministerium für Verkehr, Bau, und Stadtentwicklung (project no. 03WWBE061) is gratefully acknowledged.

Competing interests

The authors declare no competing interests.

References

1. **York, W.S., A.G. Darvill, M. McNeil, T.T. Stevenson, and P. Ablersheim.** 1985. Isolation and characterization of plant cell walls and cell wall constituents. Methods Enzymol. *118*:3-40.
2. **Doyle, J.J. and E.E. Dickson.** 1987. Preservation of plant samples for DNA restriction endonuclease analysis. Taxon *36*:715-722.
3. **Pyle, M.M. and R.P. Adams.** 1989. In situ preservation of DNA in plant specimens. Taxon *38*:576-581.
4. **Murray, M.G. and J.W. Pitas.** 1996. Plant DNA from alcohol-preserved samples. Plant Mol. Biol. Rep. *14*:261-265.
5. **Flournoy, L.E., R.P. Adams, and R.N. Pandey.** 1996. Interim and archival preservation of plant specimens in alcohols for DNA studies. BioTechniques *20*:657-660.
6. **Stowers, C.C. and E.M. Boczko.** 2007. Reliable cell disruption in yeast. Yeast *24*:533-541.
7. **Scherrer, R., L. Louden, and P. Gerhardt.** 1974. Porosity of yeast cell wall and membrane. J. Bacteriol. *118*:534-540.
8. **Ehwald, R., P. Heese, and U. Klein.** 1991. Determination of size limits of membrane separation in vesicle chromatography by fractionation of polydisperse dextran. J. Chromatogr. A *542*:239-245.
9. **Jeschke, A., D. Cech, and R. Ehwald.** 1991. Chromatographic fractionation of nucleic acids using microcapsules made from plant cells. J. Chromatogr. A *585*:57-65.
10. **Selisko, B. and R. Ehwald.** 1993. Entrapment of dextran in plant cell capsules by reversible change of cell wall permeability. J. Biochem. Biophys. Methods *27*:311-325.
11. **Wehr, C.W. and L.W. Parks.** 1969. Macromolecular synthesis in *Saccharomyces cerevisiae* in different growth media. J. Bacteriol. *98*:458-466.

Received 16 July 2009; accepted 2 July 2010.

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