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Short communication

Disrupting *Escherichia coli*: A Comparison of Methods

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The often-encountered problem of disrupting bacteria for the purpose of extracting soluble protein has generated various methods. Many require specialized equipment. Very often, especially during preliminary studies, investigators need a simple, fast, and inexpensive method for cell disruption that preserves biological activity. This paper compares some simple and inexpensive methods for cell disruption, such as bead-vortexing, freezing-thawing, French pressing, and sonication. It also provides some tips to increase protein yield and preserve biological activity. If performed under optimal conditions, bead-vortexing gives protein yields that are comparable to French pressing and sonication. It also preserves the activities of labile enzymes and releases periplasmic enzymes. Vortexing with glass beads appears to be the simplest method for cell disruption.

Keywords: *E. coli*, Cell disruption, Protein yield, Enzyme activity

Introduction

E. coli is widely used as a model organism for the study of various aspects of cell metabolism. In addition, *E. coli* is often used for the production of recombinant proteins. The first step in recovering intracellular proteins is cell disruption (Song and Jacques, 1997). Various devices for cell disruption by ultrasonication, shearing, or bead milling are available. These methods are an effective means of disintegrating *E. coli*, but they require special, and sometimes, unavailable equipment. In many cases (especially during preliminary studies), a simple, inexpensive, and fast method for cell disruption, which would preserve the biological activity in the crude extract and give a sufficient protein yield, would be helpful.

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Abbreviations: CuZnSOD, Cu and Zn-containing superoxide dismutase; DHQ synthase, dehydroquinate synthase

We compared simple and inexpensive methods for cell disruption and also delineated some optimizations in order to increase the protein yield and preserve biological activity.

Materials and Methods

Cells The strains of *E. coli* used in these experiments were as follows: AB1157, parental; JI132 *sodAsodB* (Imlay and Linn, 1987); and pLK1646pGS57, *fumA fumC*, and overproducing fumarase A (Woods et al., 1988). Fumarase A was measured in strain pLK1646pGS57. CuZnSOD and aconitase were assayed in JI132 only.

The cells were grown to mid-log phase ($A_{600\,nm}$ ~0.8-1.2) in either LB or M9 salt medium that was supplemented with the required amino acids, 0.3 g/L thiamin, 0.3 g/L pantothenate, and 0.2% glucose (Imlay and Linn, 1987). Aromatic amino acids were omitted for the DHQ synthase assay. For assaying glycerol kinase, the cells were grown in 1% casamino acids that contained 20 mM glycerol. For CuZnSOD, the cells were grown to stationary phase ($A_{600\,nm}$ = 2.1-2.2).

The cells were washed 3 times with a 50 mM potassium phosphate buffer, pH 7.4, and resuspended in the same buffer to a final $A_{600\,nm}$ of 10.0. When the suspensions with optical density of less than 10.0 were used, the yield was multiplied by the dilution coefficient in order to give mg of protein per 1.0 ml suspension having $A_{600\,nm}$ of 10.0.

Cell disruption French pressing was performed on a Spectronics Instruments press that was equipped with a mini-cell. The pressure was adjusted to 900 psi, corresponding to 20,000 psi in the mini-cell. The flow rate was 18-20 drops/min.

Sonication was performed on a Torbeo Ultrasonic processor that was equipped with a micro tip. The cells were intermittently sonicated on ice for 30 s with 30 s allowed for cooling. The total sonication time was 2, 4, or 6 min.

For freezing and thawing, 1.0 ml cell suspension in a 15 ml glass tube was either immersed in liquid nitrogen for 40 s, or kept in a freezing compartment of a refrigerator (–24 ~ –28°C) for 10 min. Complete freezing of the 1.0 ml sample in the second case required about 7 min. Frozen samples were then placed in a 30°C water bath for thawing.

Bead-vortexing was performed in a cold room on a Stuart

Scientific Autovortex Mixer SA2 at 3,000 rpm with 0.2-1.5 g glass beads/ml (0.1 mm diameter, Cole Parmer).

In the case of oxygen-sensitive enzymes, the tubes were purged with Ar and kept under Ar if the disruption method permitted.

All of the manipulations were performed at 4°C. The tubes were kept on ice. Cell lysates were centrifuged for 15 min at 20,000 × *g* at 4°C. They were assayed for superoxide dismutase (McCord and Fridovich, 1969), fumarase (Liochev and Fridovich, 1993), aconitase (Gardner and Fridovich, 1991), DHQ synthase (Hasan and Nester, 1978), glycerol kinase (Hayashi and Lin, 1967), and total protein (Lowry *et al*., 1951). To obtain the total aconitase and fumarases activities, the enzymes were reactivated in crude extracts as described by Gardner and Fridovich (1992), and Liochev and Fridovich (1993), respectively.

Results

Protein yield In order to monitor cell disruption, the total protein (mg/ml) and activities of several enzymes were assayed in the extract.

In order to learn the optimal conditions for achieving the maximal release of proteins, we varied the number of passes (French Press), total duration of treatment (sonication and bead-vortexing), or number of cycles (freezing-thawing). Fig. 1 summarizes the results for AB1157. Similar results were obtained for the other strains. Protein release did not depend on the strain or the growth medium.

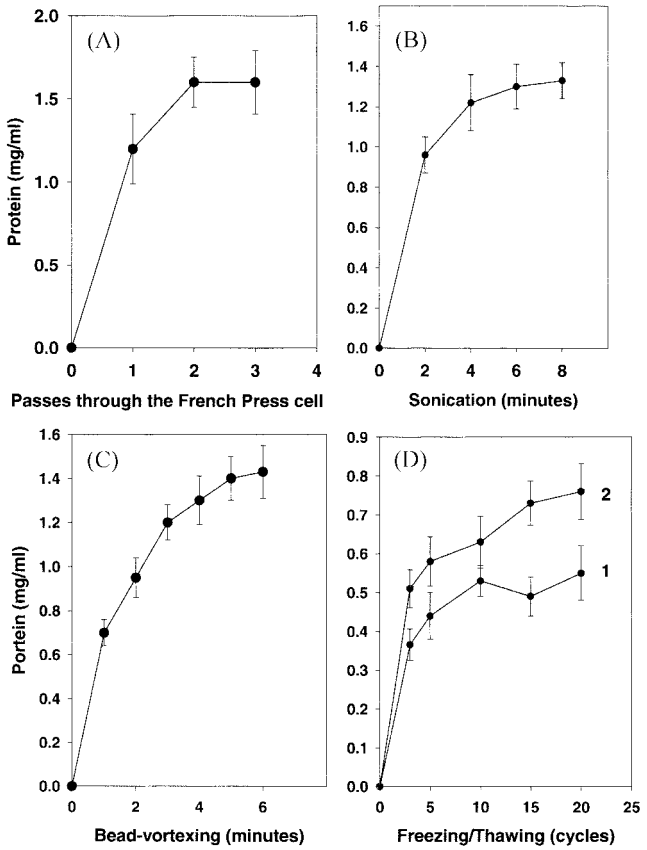

Fig. 1 Protein yield. One ml portions of *E. coli* cell suspension were disrupted by French pressing (panel A), ultrasonication (panel B), vortexing with beads (panel C), or freezing/thawing (panel D). Panel D line 1 represents the protein yield at a fast-freezing rate (liquid nitrogen); line 2, a slow-freezing rate (~ 5°C/min). Error bars show S.E. (n=5)

Protein release by freezing-thawing was strongly dependent on the density of the suspensions. As a standard, we used suspensions with a density that corresponded to $A_{600\,nm} = 10$. Suspensions with a density of $A_{600\,nm} = 15$ or higher gave almost the same amount of total protein as the suspensions with a density of $A_{600\,nm} = 10$. Increasing the number of freezing-thawing cycles (data not shown) could not compensate for this decreased yield. The optimum for obtaining the highest protein yield was $A_{600\,nm}$ 4.0-5.0. Another factor that affected the protein recovery was the rate of freezing. Slow freezing in the freezing compartment of a refrigerator (~5°/min) released more protein than fast freezing in liquid nitrogen (Fig. 1D, line 2).

When the cells were disrupted by vortexing with glass beads, the major factor that influenced the yield was the ratio of glass beads/cell suspension volume. For suspensions with $A_{600\,nm} = 10$, the optimal ratio appeared to be ~1.0 g of glass beads per ml of final cell suspension. Since this bead amount makes a thick slurry, the crude extract was collected with an automatic pipette. To prevent the pipette tip from plugging, the beads were spread on the walls of the tube by gentle rotation of the inclined tube before the crude extract was sucked out. The shape and size of the tube in which the suspension was vortexed also affected protein recovery. For 1.0-2.0 ml of cell suspension, round-bottom glass centrifuge tubes with ~15 mm internal diameter gave the best results. In this case, up to 7 tubes can be vortexed at the same time if bound tightly together with rubber bands. The variations among the tubes did not exceed 10%.

Activity The enzymes that were chosen as markers for biological activity differed in respect to cellular localization and stability.

CuZnSOD is periplasmic and relatively unstable (Benov *et al.*, 1997). Its activity in the crude extract was strongly dependent on the manner of cell disruption. The highest activity was obtained when the cells were disrupted by bead-vortexing (Table 1), followed by French pressing. Release of the enzyme by French pressing depended on the density of the cell suspension. CuZnSOD activity was almost completely absent if $A_{600\,nm}$ of the cell suspension was less than 10. No CuZnSOD activity was detected in the crude extracts that were obtained by sonication and freezing-thawing.

Fumarase A and aconitase are inactivated by oxygen/superoxide (Gardner and Fridovich 1991; Liochev and Fridovich, 1993). The fumarase and aconitase activities were highest in crude extracts that were obtained by French pressing and bead-vortexing (Table 1). Prolonged sonication and many cycles of freezing/thawing markedly inactivated

Table 1. Enzyme activities

Method of cell disruption	Enzymes assayed				
	CuZnSOD[1] (U/mg)	Fumarase[2] (U/mg)	Aconitase[1] (mU/mg)	Glycerol kinase (U/mg)	DHQ synthase[3] (mU/mg)
French press	1.3 ± 0.2	0.32 ± 0.04	23 ± 3	0.08 ± 0.02* 0.87 ± 0.13**	21.7 ± 3.4
Sonication	N.D.	0.17 ± 0.06	17 ± 2	0.09 ± 0.01* 0.67 ± 0.32**	20.9 ± 4.1
Bead-vortexing	3.7 ± 0.3	0.41 ± 0.05	26 ± 4	0.07 ± 0.02* 0.94 ± 0.21**	21.3 ± 2.8
Freezing-thawing	N.D.	0.24 ± 0.04	10 ± 3	N.D.* 0.89 ± 0.9**	19.7 ± 3.2

Cells were disrupted at conditions giving maximal protein yield. The mean ± S.E.M. is presented (n = 5).
[1]Assayed in JI132
[2]Assayed in pLK1646pGS57
[3]Cells were disrupted in the presence of 1 mM $CoCl_2$
*Grown on glucose
**Grown on glycerol

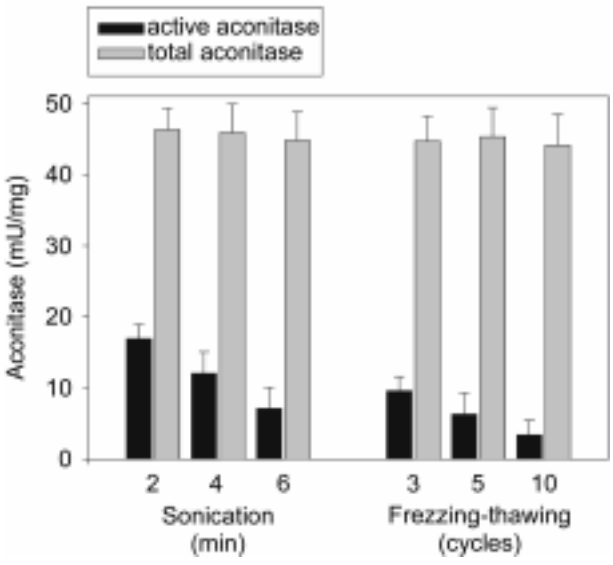

Fig. 2. Inactivation of aconitase Ultrasonication or freezing/thawing disrupted the cells. Aconitase was immediately assayed. To obtain the total aconitase activity, the enzyme was reactivated anaerobically. Error bars represent S.E. (n=5).

aconitase (Fig. 2). Similar results were obtained for fumarase A (data not shown). Disrupting the cells under a flow of Ar prevented the inactivation to a great extent. However, not all of the methods that were tested could be performed under argon. Bead-vortexing appeared to be most suitable for keeping the samples in an anaerobic atmosphere. Cell suspensions that contained glass beads were purged with argon, and the tubes were tightly closed. Therefore, all of the manipulations were performed under anaerobic atmosphere.

Glycerol kinase is an inducible enzyme whose activity is very low if glucose is present in the medium. Such a low activity could be detected only in crude extracts that were obtained by French pressing, ultrasonication, or bead-vortexing, but not by freezing-thawing (Table 1).

DHQ synthase activity depended on the presence of Co^{2+}. The addition of $CoCl_2$ to 1 mM before cell disruption increased the specific activity by ~20%. All of the methods were equally efficient in releasing DHQ synthase (Table 1).

Discussion

The often-encountered problem of disrupting bacteria for the purpose of extracting soluble protein has generated several methods. Some of these call for specialized equipment. In this paper, the most frequently used methods have been systematically compared and optimized.

Multiple cycles of freezing-thawing yielded approximately half the amount of solubilization that could be achieved either by French pressing or sonication. Maximal release of protein was obtained with suspensions having $A_{600\,nm}$ of 5 or less, and a freezing rate that did not exceed 10°C/min. Even if performed under optimal conditions, freezing/thawing did not appear to be suitable for obtaining a high protein concentration, or releasing labile enzymes.

Bead-vortexing is preferable, not only when a high protein concentration is needed, but also when proteins easily lose activity. Because the samples can be kept under an anaerobic atmosphere and temperatures very close to 0°C, the activities are maximally preserved. With respect to protein yield, bead-vortexing is comparable to French pressing and sonication. To achieve a maximal release of proteins, the ratio of glass beads to cell suspension should be kept close to 1 : 1 (w/v). Under these conditions and $A_{600\,nm}$ ~10.0, 6 min of vortexing at 3,000 rpm in a round-bottom glass centrifuge tubes gives the highest protein yield. In addition, this method is suitable for extracting periplasmic enzymes. Because of its simplicity, and

since multiple samples can be processed in parallel, vortexing with glass beads appears to be the simplest method for cell disruption when specialized equipment is unavailable.

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