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Facile and Gentle Method for Quantitative Lysis of *Escherichia coli* and *Salmonella typhimurium*

SARA CRABTREE AND JOHN E. CRONAN, JR.*

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

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Garrett et al. (Mol. Gen. Genet. **182**:326–331, 1981) constructed strains of *Escherichia coli* harboring derivatives of plasmid pBR322 that carry the lysis genes (*S*, *R*, and R_z) of phage lambda. The plasmid construction placed the genes under control of the lactose operon operator-promotor (and thus of *lac* repressor). Induction of *E. coli* strains carrying these plasmids resulted in rapid lysis of the culture unless the *S* gene was defective, in which case the cells grew normally. A freeze-thaw treatment of induced cells carrying an S^- plasmid gave quantitative lysis of either *E. coli* or *Salmonella typhimurium* cells under exceptionally gentle conditions. The method was equally effective on exponential phase cells and stationary phase cells and was readily extended to a large number of independent cultures.

Although many methods are available to disrupt *Escherichia coli* and *Salmonella typhimurium*, each has its disadvantages. Disruption by sonic oscillation is difficult to reproduce and can lead to enzyme inactivation by heating and surface denaturation. Disruption by hydrodynamic shearing using a pressure cell largely avoids these problems but is difficult to apply to multiple samples. Spheroplasting with lysozyme-EDTA treatment, although a very useful technique, can lead to artifacts owing to the properties of the reagents. Lysozyme possesses an intrinsic protease activity that cleaves some proteins (18) and can also cause artifactual association of acidic macromolecules by virtue of its net positive charge (18, 19). The chelator, EDTA, is needed both to allow the lysozyme access to the peptidoglycan and to destabilize the spheroplasts to osmotic lysis (27). However, EDTA will disrupt complexes linked by divalent cations as well as inactivate metalloenzymes. Spheroplasts can be gently lysed in the presence of divalent cations with nonionic detergents (11) but the detergents may also create artifacts (3). Other methods such as induction of a lysogenic prophage (4) or treatment with inhibitors of cell wall biosynthesis require active metabolism of the cells and thus are not suited to fully grown cultures. In this note, we show that freeze-thaw treatment of cells containing the *R* and R_z proteins of bacteriophage λ results in essentially quantitative lysis under extraordinarily mild conditions. This method is readily applicable to large numbers of small samples as well as to large and very concentrated samples.

Bacteriophage λ carries three genes required for cell lysis, *S*, *R*, and R_z. Garrett et al. (8) have inserted this portion of the λ genome into a derivative of plasmid pBR322 in such a manner as to put the expression of these genes under control of the lactose operon operator-promotor. Induction by addition of isopropyl thiogalactoside (IPTG; a gratuitous inducer of the lactose operon) to cells carrying a plasmid with functional copies of *S*, *R*, and R_z results in cell lysis (1, 8, 9). However, if the *S* gene is defective, the cells do not lyse despite the accumulation of high intracellular levels of the *R* and R_z products (1, 8, 9). Such cells do lyse when frozen and thawed (see below) or when exposed to $CHCl_3$ (8, 21).

The current interpretation (1, 7, 9, 25, 26) of the mechanism of bacteriophage λ-induced lysis is that the *R* and R_z proteins are enzymes that degrade the peptidoglycan layer of the cell envelope. The *R* gene product is a transglycosidase, whereas the R_z protein may be an endopeptidase (1, 8, 9). These proteins are inactive in cell lysis unless the *S* gene function is present (8, 19). A recent working model for *S* gene function, for which there is some support (24), is that the *S* gene product may form a pore in the cytoplasmic membrane through which the small (molecular weight, 17,000) *R* and R_z proteins must pass to reach their peptidoglycan substrate. The freeze-thaw and $CHCl_3$ treatments are thus believed to mimic the action of the *S* gene product by disruption of cytoplasmic membrane, thus allowing the *R* and R_z proteins access to the peptidoglycan (7, 20, 24).

In our work, the pJH2 plasmid of Garrett et al. (8) was introduced into the host strain by transformation. The efficiency of transformation was much increased if the strain also carried a mutation ($lacI^q$) in the promotor of the *lacI* (repressor) gene resulting in overproduction of the *lac* repressor (16, 22) (Garrett and co-workers [8, 9] had routinely included the $lacI^q$ mutation in their strains). This character can be readily introduced on an F $lacI^q$ *lacZ*::Tn*5* episome by conjugation and selection for the neomycin (or kanamycin) resistance encoded by Tn*5*. It should be noted that the level of *lac* repressor encoded by a normal *lacI* gene is insufficient to repress expression of the lysis gene by plasmid pJH2 since the number of copies of the *lac* operator carried by the multicopy plasmid exceeds the number (5 to 10) of repressor molecules (22). The presence of the F $lacI^q$ seemed essential for introduction of pJH2 into *S. typhimurium*. In wild-type (lac^+) *E. coli* strains, the requirement for $lacI^q$ was not absolute, but the efficiency of transformation was abnormally low probably due to the fragility of the transformed cells (see below).

Strains carrying pJH2 and $lacI^q$ grew normally even when the synthesis of the *R* and R_z proteins was fully induced (8, 9). We found normal growth to proceed for a very large number of cell doublings; for example, colony formation was normal in the presence of IPTG (data not shown). However, if these induced cells were subjected to a cycle of freezing and thawing, quantitative lysis was obtained (Table 1). To assay lysis, we used the release of enzyme activity: β-galactosidase for *E. coli* and glucose-6-phosphate dehydrog-

* Corresponding author.

enase for *S. typhimurium*. These enzymes are known to be localized in the cytosol and not in the periplasm (13).

The release of β-galactosidase by freeze-thaw treatment of induced cells carrying the pJH2 plasmid was quantitative (Table 1). The amount of β-galactosidase activity obtained was indistinguishable from that obtained by French press treatment and greater than the level given by a treatment with a mixture of toluene and sodium dodecyl sulfate reported to give quantitative release (20) of β-galactosidase from *E. coli*. Moreover, further treatment of the frozen and thawed cells by French press or toluene-sodium dodecyl sulfate gave no further release of activity. The addition of thiodigalactoside, a specific inhibitor of lactose transport (17), had no effect on the activity obtained, thus demonstrating that none of the β-galactosidase activity observed was due to transport of the substrate (*o*-nitrophenylgalactoside) into unbroken cells followed by hydrolysis. The freeze-thaw treatment was equally effective on logarithmic phase and stationary phase cells and could be done either on cell pellets obtained by centrifugation (Table 1) or on cells suspended in the original culture medium (data not shown). Entirely analogous results were obtained with *S. typhimurium* in which glucose-6-phosphate dehydrogenase release was assayed (Table 1).

Several points must be noted when using the pJH2 plasmid. First, the growth medium cannot contain a strongly catabolite repressing compound such as glucose since this will repress *R* and R_z gene expression. Second, the *S* gene mutation (*S*am7) carried on the pJH2 plasmid is suppressed only by the *supF* (*tyrT*) repressor of *E. coli* (15, 24) and presumably by the analogous *S. typhimurium* suppressor (26) but not by other suppressors. Although the *supF* suppressor is infrequently found in *E. coli* K-12 strains (15), its presence should be suspected if lysis of the culture occurs after addition of IPTG. Another potential problem also seems to be the result of the *S*am7 mutation. As mentioned above, it is difficult to introduce the pJH2 plasmid into *E. coli* strains carrying only a normal ($lacI^+$) level of *lac* repressor. Moreover, those strains which received the plasmid grew poorly (at about 20% of the normal rate), and a portion of the cells lysed as demonstrated by the presence of large amounts of β-galactosidase in the growth medium. This problem may be more acute in the *lac S. typhimurium* because we were repeatedly unable to introduce the plasmid into a strain lacking the F $lacI^q$ plasmid (a few small Amp^R colonies formed but failed to grow upon replating).

At first glance, the behavior of strains carrying pJH2 in the absence of repressor overproduction seemed to conflict with the normal growth observed in the presence of IPTG of strains carrying pJH2 and $lacI^q$. However, there are quantitative distinctions between these situations. It first should be noted that a $lacI^+$ strain carrying pJH2 is expected to be functionally *lacI* owing to titration of the *lac* repressor by the multiple copies of the *lac* operator present, due to the multicopy nature of the plasmid (22). This has been shown directly (data not shown). Second, there is a quantitative difference between $lacI^+$, *lacI*, and $lacI^q$ strains of *E. coli* in that the levels of lactose operon expression in $lacI^q$ strains are considerably lower than those given by the other strains in the presence of IPTG (10, 16). These observations have been explained as the result of a residual affinity of the repressor-inducer complex for the *lac* operator (10); therefore, those strains having higher levels of repressor have a lower maximal rate of *lac* operon expression (10, 16). We thus assume the differences we observed in cell growth were due to the differences in the amounts of the amber fragment of *S* protein produced in the $lacI^+$, $lacI^q$, and *lacI* strains.

TABLE 1. Lysis of *E. coli* K-12 and *S. typhimurium* LT2

Method of disruption	β-Galactosidase for *E. coli*[a] (U/mg of cell protein)	Glucose-6-phosphate dehydrogenase *S. typhimurium*[b] (U/mg of cell protein)
Logarithm phase cells		
No treatment[c]	<0.01	
French press	2.27	41.3
Freeze-thaw	2.18	41.6
Freeze-thaw followed by French press	2.05	32.4
Toluene-SDS	1.09	
Freeze-thaw plus toluene-SDS	1.92	
Stationary phase cells		
No treatment[c]	<0.01	3.2
French press	1.52	27.4
Freeze-thaw	1.49	45.4
Freeze-thaw followed by French press		44.8

[a] The *E. coli* K-12 strain was CQ (8), a derivative of strain CSH57 carrying both $lacI^q$ and $lacZ^+$ on the chromosome. Strain CQ and a derivative carrying plasmid pJH2 [CQ(pJH2) were the generous gift of R. Young (7, 8). The logarithmic phase experiments were done on strain CQ(pJH2), using cultures grown to about 5×10^8 cells per ml in M9 medium (14) with 0.4% glycerol as the carbon source. The medium also contained the nutrients required by these polyauxotropic strains and sodium ampicillin (5 μg/ml). IPTG (1 mM) was added when the cultures reached 2×10^8 cells per ml. A sample of the induced culture (0.6 ml) was centrifuged, and the drained pellet was frozen in solid carbon dioxide-ethanol (or liquid nitrogen) and then thawed for 15 min at room temperature. After a second freeze-thaw, a few crystals of DNase and RNase were added to decrease viscosity. The lysed cells were then suspended in 3 ml of M9 medium containing chloramphenicol (25 μg/ml). β-Galactosidase activity was assayed as previously described (16) (with the omission of the cell lysis step). In some cases, thiodigalactoside (5 mM) was included to inhibit the lactose permease (16) and thus prevent expression of β-galactosidase activity present in intact cells. The stationary phase cells were treated identically, except that the cultures were grown to stationary phase (about 3×10^9 cells per ml) and were harvested after no turbidity increase was observed for 0.5 to 1 h. For French press treatment, the cell pellets (with or without freeze-thaw treatment) were suspended in M9-chloramphenicol and passed through the cell twice at 16,000 lb/in^2 before β-galactosidase assay. In some cases, disruption by a mixture of toluene and sodium dodecyl sulfate (SDS) (19) was used. One unit of β-galactoside activity was 1 nmol of *o*-nitrophenol produced per min at 25°C. Similar results were obtained in five other experiments with strain CQ and with other pJH2 $lacI^q$ strains of *E. coli* K-12.

[b] The *S. typhimurium* LT2 strain was CY360. Strain CY360 was constructed by first introducing the F $lacI^q$ *lacZ*::Tn*5* episome from *E. coli* WFK (from R. Young [8]) by mating with the restriction-negative LT2 strain TL156 (5). The resulting strain was then transformed with plasmid pJH2. Plasmid preparation by alkaline lysis of strain CQ(pJH2) and transformation were done as described by Maniatis et al. (14), except that 20 mM $MgCl_2$ was added both to the growth medium and all buffers as recommended by Hanahan (12). Strain CY360 was grown and treated as the *E. coli* strains, except that the suspending buffer was 10 mM Tris-hydrochloride (pH 7.8) containing 10 mM $MgCl_2$ and 1 mM dithiothreitol. Glucose-6-phosphate dehydrogenase was then assayed as described by Fraenkel and Levine (7). A unit of dehydrogenase activity was 1 pmol of NADPH formed per min at 25°C.

[c] The cells were removed by centrifugation before enzyme assay.

The N-terminal half of the protein is thought to have some residual activity (9, 24), and thus the level of production could account for the poor growth and lysis of the *lacI*$^+$ (phenotypically *lacI*) strains carrying pJH2 observed in the absence of IPTG.

Plasmids carrying the lambda *R* and R_z genes should be useful in a number of applications. In this laboratory, we have used the pJH2 plasmid (i) to produce cell extracts only three- to fourfold less concentrated than the cytosol to examine the effect of protein concentration on weak complexes among fatty acid biosynthesis proteins (S. Crabtree, unpublished data), (ii) to lyse multiple small stationary-phase cultures grown in microtiter dishes in screening for clones deficient in various lipid metabolic enzymes (J. Lampi, unpublished data), and (iii) to lyse cells infected with nonsense mutants of phage PR4 (6). H. Leibke (personal communication) has used plasmid pJH2 for cell lysis in the isolation of an EDTA-sensitive ribonucleoprotein complex from *E. coli*. Use of strains carrying pJH2 might also be a useful modification of various methods used to screen for bacterial mutants deficient in specific enzymes such as those of Tabor et al. (23) and Bulawa et al. (2). In addition, preliminary results indicate that inner and outer membranes can be prepared by osmotic lysis of *E. coli* strains carrying pJH2 after induction with IPTG. However, the purity and yield of the membrane fractions do not seem appreciably greater than those obtained by conventional techniques (data not shown).

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