BIOTECHNOLOGY TECHNIQUES
Volume 9 No.7 (July 1995) pp.477-480
Received as revised 16th May

RELEASE OF CHLORAMPHENICOL ACETYL TRANSFERASE FROM RECOMBINANT *ESCHERICHIA COLI* BY SONICATION AND THE FRENCH PRESS

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Summary

The release of chloramphenicol acetyl transferase (CAT) from a recombinant *Escherichia coli* strain by ultrasonication and the French press was compared. French pressing disrupted all cells in suspension whereas only a fraction of the cells was disrupted following sonication. The level of CAT released was highest when cells were totally disrupted. Additional treatment with the detergent Triton X-100 was necessary to maximize CAT recovery, presumably due to association of CAT with cellular debris.

Introduction

Meaningful comparison of bacterial expression systems relies on a disruption method that completely releases the intracellular reporter protein without degradation. Techniques available for laboratory-scale disruption have previously been reviewed (Hughes *et al.*, 1971), and include thermal, enzymatic, chemical and mechanical methods. The most efficient method for cell disruption will totally release the desired product without degrading or denaturing it. Such methods are routinely used. However, an evaluation of any method's effectiveness is seldom undertaken.

Chemical treatment with acetone and SDS can release intracellular proteins from *Escherichia coli* in equivalent amounts to sonication and bead mill agitation (Bhaduri and Demchick, 1983). The presence of SDS makes this method unsuitable for the preparation of extracts for enzyme assays, because enzyme activity may be inhibited. For example, detergents such as Triton X-100 and SDS inhibit CAT enzyme activity (Lu and Jiang, 1992). Combined chemical and enzymatic treatments are also effective but results depend on the

growth phase (Dean and Ward, 1992). This complicates meaningful comparison of promoter strength for organisms in different growth phases.

Mechanical disruption methods such as ultrasonication and French pressing are often used at laboratory scale to overcome the problems of incomplete and variable release associated with chemical and enzymatic disruption methods. However, sonication has also been reported to inactivate enzymes (Desai, 1968) and result in variable release (Desai, 1968; Feliu and Villaverde, 1994). Furthermore, the efficiency of the sonication treatment is affected by sample volume (Feliu and Villaverde, 1994) and power output (Caldeira and Cabral, 1994). A comparison of the methods showed that the French press was more efficient at releasing proteins than sonication (Schmitt, 1976). However, it was not established that complete disruption was obtained.

In this study, we have compared the effectiveness of sonication and the French press for cell disruption and release of CAT. Actual cell disruption is determined and compared with measurements of total CAT release. The effect of adding the detergent Triton X-100 following disruption by sonication and French pressing is examined.

Materials and Methods

Bacterial Strain. *E.coli* JM101 (F' traD36 lacI^{q}Δ(lacZ)M15 proA$^+$B$^+$ /supE thi Δ(lac-proAB) TonA).
Plasmid. *cat* was encoded on a pBR322-based plasmid (Williams and Manning, 1991) under control of the IPTG-inducible *trc* promoter. The plasmid is designated pCT102.
Media. M9 minimal medium, pH = 7.5, supplemented with thiamine, 40 μg/ml, and Ampicillin, 100 μg/ml.
Method. Minimal medium, 200 ml, was inoculated with a single colony of JM101, transformed with plasmid pCT102, and cultivated at 37°C. At OD(600 nm) = 0.8, CAT expression was induced by addition of IPTG to 0.4 mM. After induction for 3 h, the culture was washed and resuspended in buffer (10 mM Tris, 1 mM EDTA).
Disruption of cells. Cell suspensions (2ml) were sonicated in 10 ml tubes using a Branson Sonifier, Model B-15. Other suspensions (10ml) were disrupted using a French press operated at 100 MPa. Disrupted samples were treated with detergent (Triton X-100, supplied with the CAT-ELISA kit), except where indicated. Cell disruption was quantified using an Applied Imaging disc centrifuge (Middelberg, 1992).
CAT assay. Cell homogenates were centrifuged (12000 g, 4°C, 10 min) to remove cell debris. CAT protein in the supernatant was measured using a CAT-ELISA kit obtained from Boehringer Mannheim (Sydney, Australia).

Results and Discussion

Figure 1 illustrates the effect of Triton X-100 on CAT recovery from cells disrupted by French pressing. Treatment with detergent is clearly necessary to achieve maximal CAT recovery, possibly because CAT partitions to the cellular debris. The need for additional

treatment of homogenates coupled with the inhibitory effect of detergents (particularly Triton X-100) confirms that quantitative measurement of total CAT by ELISA provides a more appropriate measure of promoter strength than measurement of CAT enzyme activity.

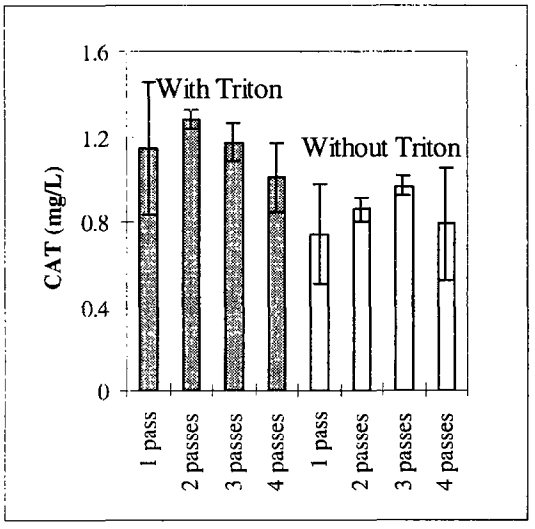

Figure 1. Effect of Triton X-100 on CAT recovery from *E. coli* cells disrupted by French pressing.

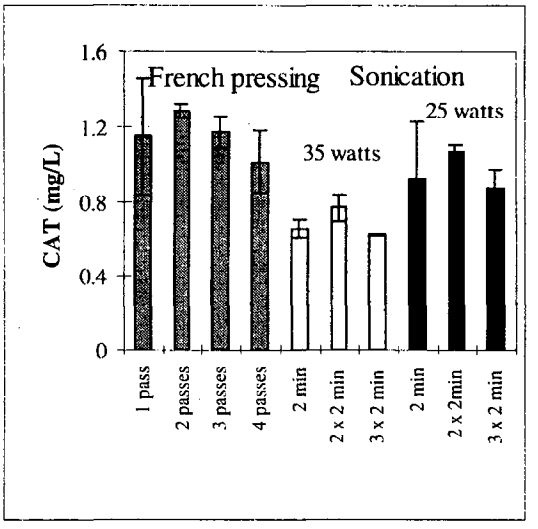

Figure 2. Recovery of CAT from *E. coli* after disruption by French pressing or sonication at 25 or 35 W. All samples were treated with Triton X-100 after disruption (refer: Materials and Methods).

Figure 2 shows a comparison of CAT recovery using sonication or French pressing, followed by treatment with Triton X-100. Maximal CAT recovery following sonication was achieved at a power level of 25 W, using two sonication cycles. Additional cycles caused decreased CAT levels. Increasing the power level did not lead to increased recovery. A possible explanation is that higher output levels lead to increased heat generation with consequent CAT degradation. Figure 2 also demonstrates that higher CAT recovery was achieved by French pressing and treatment with Triton X-100 than by sonication.

Comparison of figures 2 and 3 suggests that the comparatively low recovery of CAT following sonication may be attributable to incomplete cell breakage. The French press totally disrupted the cell wall of *Escherichia coli*, whereas only a fraction of cells was disrupted following sonication. One pass through the French press achieved 99% disruption compared with 42% disruption for a single two minute sonicator treatment. A longer sonication cycle (3x2 min) only increased disruption to 80%. Data following sonication for 2 min show that less than half of the cells were disrupted but more than half the CAT protein was released. This might be due to partial disruption where the cells appear intact but are sufficiently porous for CAT to be released. A similar observation was reported by Kaback and Deuel (1969). They found that sonication resulted in product release, but that intact and sonicated samples scanned in an electron microscope appeared identical.

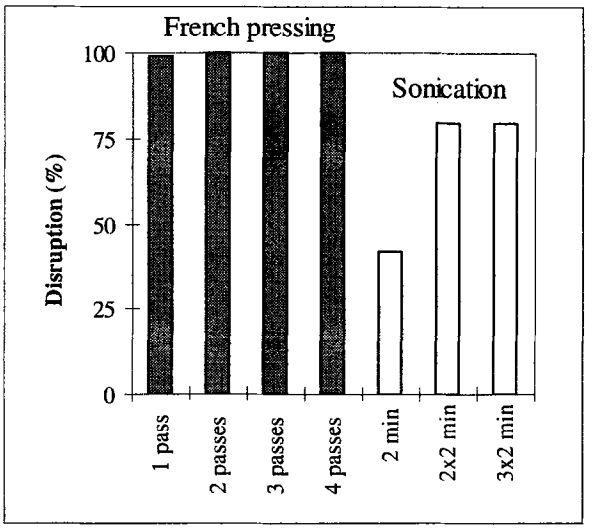

Figure 3. Disruption of *Escherichia coli.*

Conclusions

The French press provides a better disruption of cells and release of CAT than sonication. French pressing totally disrupts bacteria whereas only a fraction of cells is disrupted following sonication. However, sonication apparently makes undisrupted cells sufficiently porous for some release of CAT protein. Even when cells are totally disrupted, only a fraction of total protein is recovered. CAT presumably associates with cellular debris. It is therefore necessary to treat homogenates with a solubilising agent such as the detergent Triton X-100. The need for additional treatment coupled with the inhibitory effect of detergents confirms that quantitative measurement of total CAT provides a more appropriate measure of promoter strength.

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