

An educational model for disruption of bacteria for protein studies

Introduction

Attempts are being made to bring some of the techniques of molecular biology into high school and undergraduate college biology programmes. One example is the use of polyacrylamide gel electrophoresis to resolve proteins. Use of that method is possible owing to commercial availability of low cost electrophoresis apparatus. Often, students resolve protein by gel electrophoresis in samples supplied by the instructor. Students would learn more from such experiments if they prepared their own bacterial cell-free extracts. The methods of preparation of cell-free extract will teach students to grow and maintain bacterial cultures free of contamination and the technique for the preparation of cell-free extract to provide a rich source of cellular proteins for biochemical analysis. Traditional methods for preparing cell-free protein extracts from bacterial cells include mechanical grinding, use of lytic enzymes, use of a French Pressure Cell, agitation with glass beads, and ultrasonic treatment. Each of those methods has at least one of the following drawbacks: applicability to only a narrow spectrum of microbial species; creation of a potential biohazard problem associated with contamination of equipment and generation of aerosol; expense of equipment; limited range of quantities for which the method is suitable; difficulty; addition of extraneous proteins; and time requirements. A simple and rapid method for disruption of bacterial cells for protein studies reported by the authors (Bhaduri and Demchick, 1983) can easily be adapted for laboratory teaching courses.

Abstract

A simple and rapid method has been developed for the disruption of bacterial cells for protein studies. The method involves stepwise treatment of cells with acetone and with sodium dodecyl sulphate solution to allow extraction of cellular proteins for analysis by polyacrylamide gel electrophoresis. The method has potential for teaching. It is safe and easy for students to do and suitable for making extracts from almost any size culture, and is likely to work with any bacterial species.

Materials and methods¹

Bacterial strains

Strains of *Escherichia coli* 600, *Yersinia enterocolitica* 217, *Yersinia enterocolitica* 225, *Clostridium sporogenes* B1219, *Clostridium novyi* type 18255, and *Staphylococcus aureus* 184 were used for the preparation of cell-free extract.

Media and solutions

Brain heart infusion (Difco Laboratories) medium contained 3.7 g powder per 100 cm³ (mass/vol.) of distilled water. Phosphate-buffer saline (PBS) was prepared by adding enough 0.02 mol dm⁻³ KH₂PO₄ in 0.85 per cent NaCl (mass/vol.) to 0.02 mol dm⁻³ Na₂HPO₄ in 0.85 per cent NaCl (mass/vol.) so that the pH became 7.4. 1 per cent sodium dodecyl sulphate (SDS) is prepared by dissolving 1 g SDS in 100 cm³ (mass/vol.) of distilled water.

Method of preparation of cell-free extract

The method of preparation of cell-free extract from bacterial cells is essentially as described by the authors in an earlier paper (Bhaduri and Demchick, 1983). As in the original method, strains of *E. coli*, *Y. enterocolitica*, *C. sporogenes*, and *C. novyi* of bacterial cells (see figure 1), were grown for 24 hours at 37 °C in brain heart infusion without agitation. Cells from 20 cm³ of culture were collected by centrifugation in a Sorvall centrifuge (7000 × g, rotor SA-600) at 4 °C and washed twice by suspending in 10 cm³ of ice-cold PBS and by recentrifugation as described above. The cells were resuspended in 10 cm³ of ice-cold acetone (10 cm³ per 20 cm³ of culture), held in an ice bath for five minutes, and again collected by centrifugation in a Sorvall centrifuge (7000 × g, rotor SA-600) at 4 °C. Residual acetone was removed under a gentle stream of nitrogen from a Pasteur pipette connected with tygon tubing to a nitrogen tank. Proteins were extracted from the cell pellet by incubating with 1 cm³ of 1 per cent SDS solution (1 cm³ per 20 cm³ of culture) for 10 minutes at room temperature with occasional vigorous shaking. Cell-free extract was centrifuged in a Sorvall centrifuge (7000 × g, rotor

¹ Reference to firm or brand name does not constitute endorsement by the US Department of Agriculture over others of similar nature not mentioned.

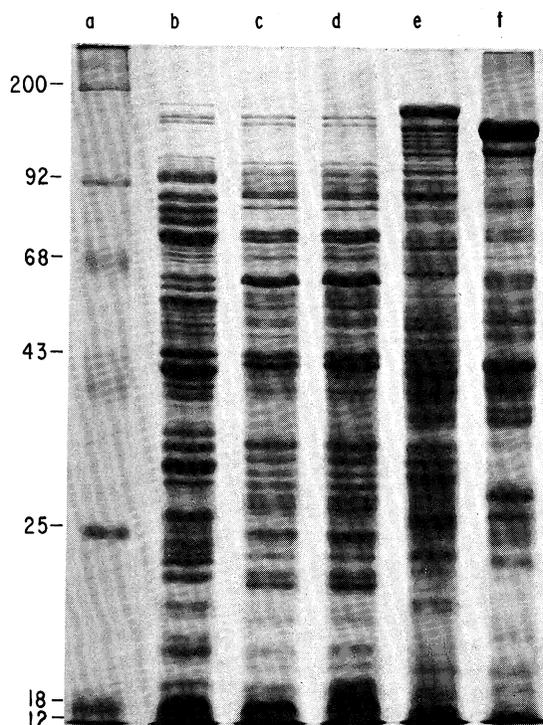


Figure 1 SDS-PAGE of proteins prepared by acetone-SDS extraction of *E. coli* 600 (lane b), *Y. enterocolitica* 217 (lane c), *Y. enterocolitica* 225 (lane d), *C. sporogenes* B1219 (lane e), and *C. novyi* type A 18255 (lane f). Lane a is marker proteins (molecular mass $\times 10^3$).

SA-600) for two minutes at room temperature to remove unbroken cells and cell debris. Proteins in the supernatants (clarified extract) were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (figure 1) (Schleif and Wensink, 1981; Bhaduri and Demchick, 1983). The organisms listed in figure 1 are recommended for this exercise.

Results and discussion

SDS-PAGE analysis of the protein extracts of different species of bacteria showed that the acetone-SDS extraction technique is applicable to a variety of bacterial species. The technique is also applicable to proteins having a wide range of molecular weights (figure 1). For teaching, the method can be made even more simple. Centrifugation can be done at any speed that yields a pellet. All the steps, including acetone treatment, can be done at room temperature. PBS washing can be omitted. The pellet can be air-dried. Times for steps and between each step are not critical.

If the length of laboratory periods makes it impossible to prepare microbial cells extracts and perform electrophoresis in the same day, clarified protein extract can be stored refrigerated for two days

or frozen for several weeks. If extracts are stored in the cold, it should be noted that 1 per cent SDS is not soluble at low temperatures. Therefore, after storage, extracts must be given time to come to room temperature to redissolve the SDS.

This method of protein extract overcomes the aforesaid limitations of other methods and can easily be adapted for teaching purpose. Protein analysis by SDS-PAGE revealed that protein compositions in extracts produced by the present method are very similar to those obtained by established methods such as ultrasonic treatment (Ames, 1974) and agitation with glass beads (Thompson and Chassy, 1981) (figure 2). The method is rapid (about one hour after

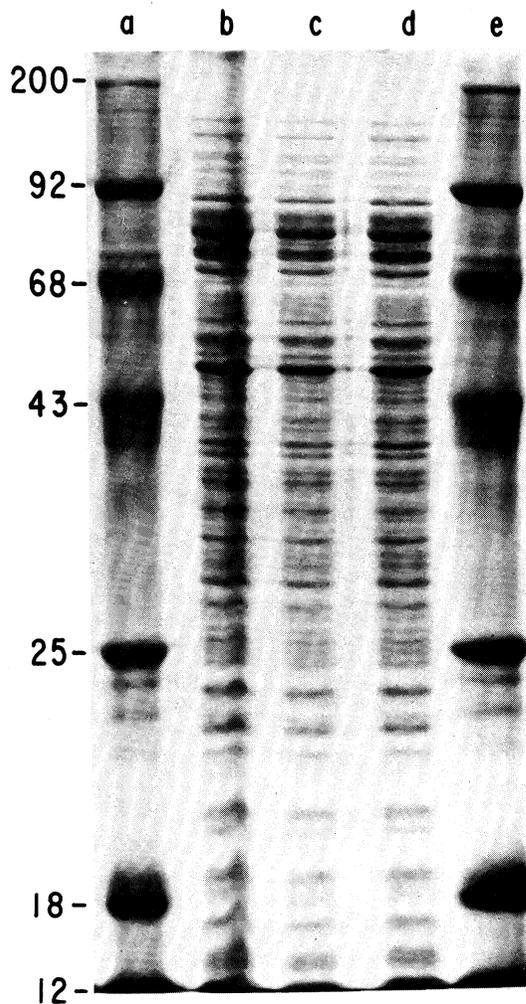


Figure 2 Comparison by SDS-PAGE of *S. aureus* 184 protein extract preparation. Protein extracts from samples were prepared by sonication (lane b), acetone-SDS (lane c), and bead agitation (lane d). Lanes a and e are marker proteins (molecular mass $\times 10^3$). (From Bhaduri and Demchick, 1983.)

Bacterial proteins Bhaduri and Demchick

the cells are grown; excluding time for electrophoresis), is well suited for classroom use, is practical for making extracts from any size culture likely to be used, and allows observance of good biosafety rules since, after the extraction procedure, all the materials can be easily decontaminated by autoclaving.

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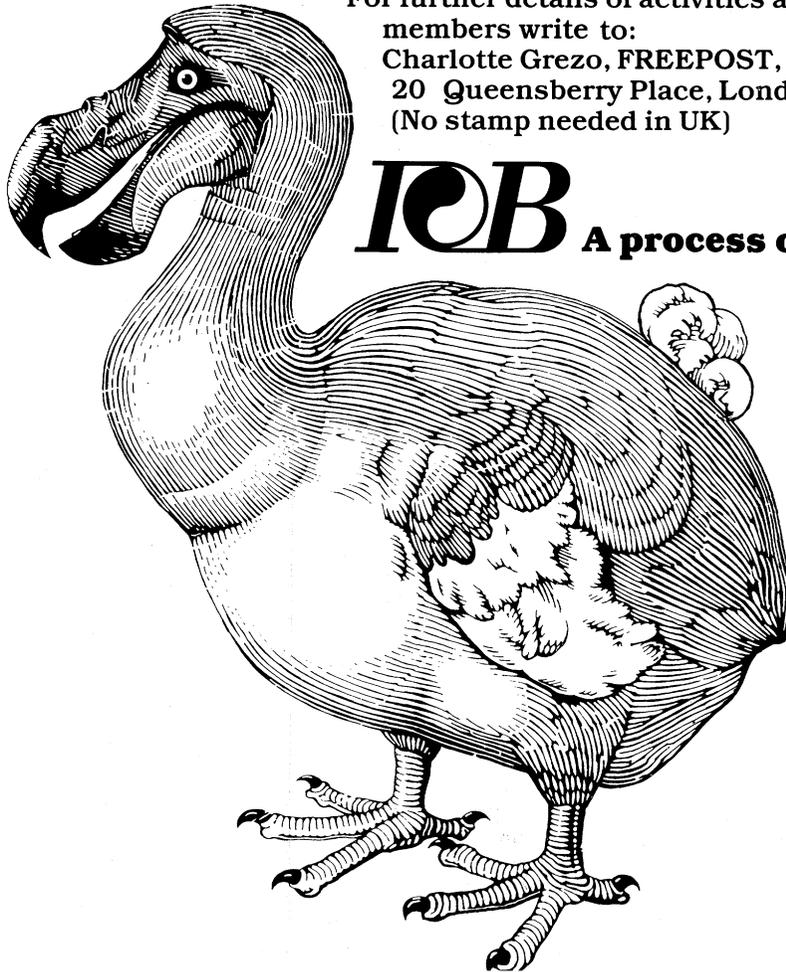
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