NOTES 4777

Simple and Rapid Method for Disruption of Bacteria for Protein Studies

SAUMYA BHADURI* AND PAUL H. DEMCHICK

Eastern Regional Research Center, Agricultural Research Center, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118

Received 16 May 1983/Accepted 3 August 1983

A simple and rapid method was developed for the extraction of proteins from both pathogenic and nonpathogenic bacteria. The method involves the treatment of cells with acetone followed by sodium dodecyl sulfate extraction of cellular proteins. Polyacrylamide gel electrophoresis revealed that the protein composition of extracts made by this method was comparable to that of extracts made by established methods, namely, sonication and agitation with beads. This technique has been successfully applied to the extraction of proteins from a wide variety of bacteria, including pathogens.

Studies of the biological, immunological, and chemical properties of cellular proteins provide valuable information pertaining to bacterial pathogenesis in humans and animals. Such studies are dependent on the efficient preparation of cell-free extracts which provide a rich source of cellular proteins. A variety of methods have been developed to prepare cell-free extracts and include both mechanical and enzymatic disruption of the bacterial cells. Mechanical disruption techniques include sonication (1, 5, 7), blending or grinding with abrasives (5, 7), agitation with glass beads (5, 7, 10), or use of the French press (7), and these procedures have been successfully used with a variety of microorganisms. Likewise, enzymatic methods including autolysis and chemical treatments have proven useful in specific instances (5, 7). The choice of specific extraction procedures is dependent on the particular species or strain of microorganism being studied, the quantity of protein required, and the type of analyses to be performed.

Preparation of cell-free extracts of pathogens presents unique difficulties. Mechanical disruption techniques are not always applicable owing to potential biohazard problems associated with contamination of equipment and generation of aerosols. Use of lytic enzymes avoids this problem; however, their addition to a cell extract may increase the complexity of subsequent protein isolations. Furthermore, many gram-positive bacteria are resistant to the action of lysozyme and other available bacteriolytic enzymes (6). The objective of this study was to develop a simple method for the extraction of cellular proteins from a variety of bacterial species, including gram-positive pathogens, that can be safely employed to produce samples suitable for biochemical analyses.

Strains of *Staphylococcus aureus, Escherichia coli*, and *Bacillus cereus* (Table 1) were grown without agitation for 24 h at 37°C in brain heart infusion (Difco Laboratories). *Clostridium botulinum* (Table 1) was grown anaerobically at 37°C for 24 h in brain heart infusion supplemented with 1% arginine to delay autolysis (4). Cells from 20 ml of culture were harvested by centrifugation (7,000 × *g*), washed twice with phosphate-buffered saline without Mg^{2+} and Ca^{2+} (3), and recentrifuged. The cells were then suspended in 10 ml of ice-cold acetone (analytical grade), allowed to stand on ice for 5 min, and collected by centrifugation (7,000 × *g*). Experiments demonstrated that acetone treatment from 5 to 30 min or the use of more acetone did not change the efficiency of protein extraction. Residual acetone was removed under a stream of nitrogen, and the proteins were then extracted by incubating with 1.0 ml of 1% sodium dodecyl sulfate (SDS) for 2 min. The acetone pretreatment was found necessary since the direct utilization of detergents did not extract cellular proteins from gram-positive bacteria.

For comparison of methods, extracts were also prepared by sonication and glass bead disruption. This was achieved by using phosphate-buffered saline-washed *S. aureus* 184 cells that were suspended in 1% SDS such that the bacte-

TABLE 1. Bacterial species used for protein extraction by acetone-SDS method

Species	Strain no.	Comment	Mg of protein extracted per g (dry wt) of cells	Source
Staphylococcus aureus	184	No enterotoxin	200	M. S. Bergdoll, Food Safety Institute, University of Wisconsin, Madison
Staphylococcus aureus	196E	Staphylococcus enterotoxins A and D	200	James L. Smith, Eastern Regional Research Center, Philadelphia, Pa.
Clostridium botulinum	Type C/AO28	Toxin C_2	150	M. W. Eklund, National Marine Fisheries Service, Seattle, Wash.
Escherichia coli	20S0	Nontoxic	225	Sheldon Finver, Eastern Regional Research Center, Philadelphia, Pa.
Bacillus cereus	5065	Emetic	200	Sheldon Finver, Eastern Regional Research Center, Philadelphia, Pa.

rial cell concentration was 20 times that of the original culture. One portion of the suspension was agitated vigorously with ca. 0.1-mm glass beads for 15 min with ice-water cooling (with Bead-Beaters; Bio-Specs, Inc.) (10). Another portion was treated ultrasonically (intermittently with ice-water cooling) as described previously by Ames (1). The extracts were clarified by centrifugation (7,000 × *g*), and the supernatants were used for protein estimation (9) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (8). Longer or more vigorous sonication or agitation with beads did not substantially increase the amount of protein extracted or change the SDS-PAGE profile.

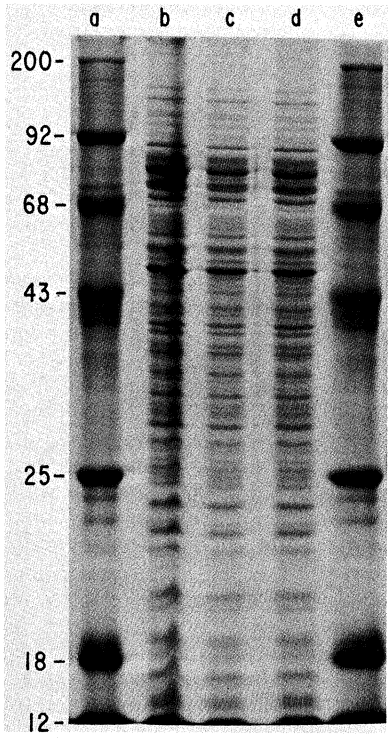

FIG. 1. Comparison by SDS-PAGE of *S. aureus* 184 protein extract preparation. Protein extracts from samples were prepared by sonication (lane b), acetone-SDS (lane c), and bead agitation (lane d). Lanes a and e are marker proteins (molecular weight × 10^3). Before electrophoresis, samples were boiled for 2 min under reducing conditions (in 0.1 M β-mercaptoethanol) and placed on 3% acrylamide stacking gel wells above a 10% acrylamide resolving gel. Electrophoresis was performed by the method of Laemmli (8). Staining and destaining procedures have been previously described (2).

The yield of protein for the acetone-SDS extraction technique and that for the sonication was 200 mg of protein per g (dry weight) of cells, whereas the bead agitation technique yielded 175 mg/g. This indicates that the efficiency of the acetone-SDS extraction technique is equivalent to that of the other techniques. The SDS-PAGE patterns of the protein extracts from the three techniques are shown in Fig. 1. The electrophoretic pattern of the acetone-SDS method (lane c) resembled those obtained by sonication (lane b) and agitation with glass beads (lane d), again indicating that the acetone-SDS method produces cell extracts equivalent to those produced by the other techniques.

The acetone-SDS method was tested with a number of other species to determine its suitability. The protein extracted per gram of dry cells from these species by this method is shown in Table 1. SDS-PAGE analysis of various amounts of the extracts obtained is depicted in Fig. 2. Adequate protein profiles were readily obtained with all of the tested species, suggesting that the extraction technique is applicable to a variety of bacterial species. The technique was applicable to proteins having a wide range of molecular weights; however, the presence of SDS would limit the use of the extraction technique for the preparation of extracts for enzyme assays.

The results indicate that the acetone-SDS extraction technique described above produces cellular protein preparations equivalent to those

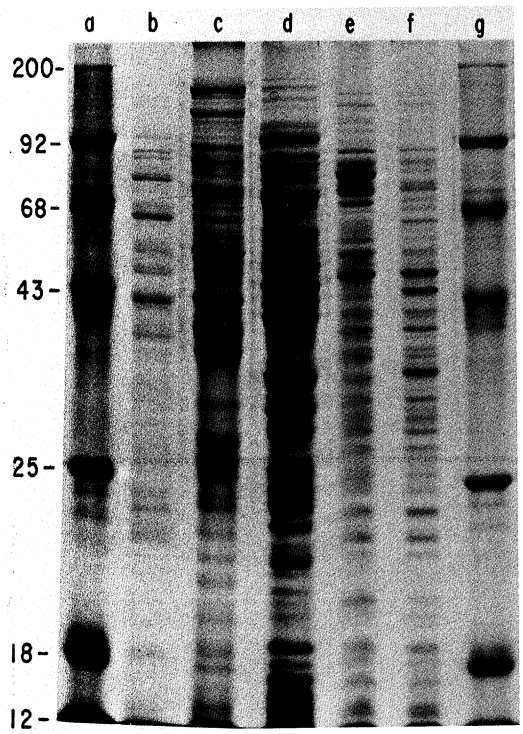

FIG. 2. SDS-PAGE of proteins prepared by acetone-SDS extraction of *B. cereus* 5065 (lane b), *C. botulinum* AO28 (lane c), *E. coli* 20S0 (lane d), *S. aureus* 184 (lane e), and *S. aureus* 196 E (lane f). Lanes a and g are marker proteins (molecular weight $\times 10^3$). Electrophoresis was carried out as described in the legend to Fig. 1.

produced by other techniques (1, 5, 7, 10). Furthermore, the present technique offers some distinct advantages since it does not require special equipment, does not involve the generation of aerosols (i.e., can be used with pathogens), does not add extraneous proteins to the samples, and can be used effectively with a variety of bacterial species. The technique appears to be an inexpensive, rapid, reproducible method for preparing large numbers of cellular protein samples regardless of pathogenicity. It was also found that the method could be scaled up or down for any volume between 1 ml to 1 liter of culture.

We thank David Schlessinger of Washington University Medical School, St. Louis, Mo., and H. M. Farrell, Jr., of the Eastern Regional Research Center, Philadelphia, Pa., for critical reading of the manuscript.

LITERATURE CITED

1. **Ames, G. F.** 1974. Resolution of bacterial protein by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249**:634–644.
2. **Basch, J. J., and H. M. Farrell, Jr.** 1979. Charge separation of protein complexed with sodium dodecyl sulfate by acid gel electrophoresis in presence of cetyltrimethylammonium bromide. Biochim. Biophys. Acta **577**:125–131.
3. **Bhaduri, S., H. J. Raskas, and M. Green.** 1972. Procedure for the preparation of milligram quantities of adenovirus messenger ribonucleic acid. J. Virol. **10**:1126–1129.
4. **Bowers, L. E., and O. D. Williams.** 1963. Effect of arginine on growth and lysis of *Clostridium botulinum*. J. Bacteriol. **85**:1175–1176.
5. **Burris, R. H.** 1972. Cell-free enzyme preparation from bacteria, p. 162–168. *In* W. W. Umbriet, R. H. Burring, and J. F. Stauffer (ed.), Manometric and biochemical techniques, 5th ed. Burgess Publishing Co., Minneapolis.
6. **Ezaki, T., and S. Suzuki.** 1982. A chromopeptidase for lysis of anaerobic gram-positive cocci. J. Clin. Microbiol. **16**:844–846.
7. **Hughes, D. E., J. W. T. Wimpenny, and D. Lloyd.** 1971. The disintegration of microorganisms, p. 1–54. *In* J. R. Norris and D. W. Ribbins (ed.), Methods in microbiology, vol. 5B. Academic Press, Inc., New York.
8. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
9. **Schleif, R. F., and P. C. Wensink.** 1981. Practical methods in molecular biology, p. 74–77. Springer-Verlag, Inc., New York.
10. **Thompson, J., and B. Chassy.** 1981. Uptake and metabolism of sucrose by *Streptococcus lactis*. J. Bacteriol. **147**:543–551.