Simple, Inexpensive Procedure for the Disruption of Bacteria

JON M. RANHAND
Laboratory of Streptococcal Diseases, The National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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Small volumes (1 to 2 ml) of bacterial cultures, with turbidities ranging from 3 to 10, were disrupted 50 to 90% (measured as a decrease in turbidity) within 2 min, by shaking them on a Vortex-type mixer in the presence of glass beads. This method of disruption was effective for cells in the following genera: Streptococcus, Lactobacillus, Staphylococcus, Bacillus, and Escherichia. After low-speed centrifugation, the resulting extract can be used as potential sources for enzymes, transforming deoxyribonucleic acids, cell walls, membranes, etc.

Most mechanical equipment that is used for the disruption of bacterial cells is expensive and requires large quantities of cells and suspending volumes. (For excellent reviews on this subject see references 3, 8, and 9.) Occasionally, smaller volumes and amounts of cells are required when a large number of cultures are screened for enzymes, transforming deoxyribonucleic acids (DNA), etc. Therefore, a simple procedure for breaking bacterial cells was devised that utilizes small volumes and existing laboratory equipment.

The results presented in this report show that simple vortexing of a suspension of bacteria, in the presence of glass beads, results in their disruption. Cell breakage was manifested as a decrease in turbidity and was confirmed by electron microscopy and by observing a loss in cell viability.

MATERIALS AND METHODS

Organisms. The organisms used in this study were as follows: Streptococcus sanguis (Wicky 4 Ery®) herein called WE4 (7), Streptococcus sanguis (FW229), Streptococcus sanguis (WE4 Rif®), Streptococcus mutans strains FA-1, AHT, BHT, and 3720, Staphylococcus aureus strain 6538P, Lactobacillus casei var. rhamnosus, Bacillus subtilis (Marburg), Escherichia coli B, and E. coli TAU. S. sanguis (WE4 Rif®) was isolated in this laboratory (6); S. sanguis (FW 229) was originally obtained from R. Pakula, University of Toronto, School of Hygiene, Toronto, Ontario, Canada; the S. mutans strains were obtained from the National Institute of Dental Research, Bethesda, Md.; S. pyogenes (K 56) was from our culture collection; S. aureus (6538P), L. casei var. rhamnosus, and B. subtilis (Marburg) were obtained from T. Theodore, this laboratory; E. coli B was obtained from K. Nugent, this laboratory; and E. coli TAU was obtained from R. Lazzarini, National Institute of Neurological Diseases and Stroke, Bethesda, Md.

Cell growth. All cultures except L. casei var. rhamnosus were grown in brain heart infusion (Difco) containing heat-inactivated (56°C, 30 min) horse serum (2.6%, vol/vol; Microbiological Associates, Bethesda, Md.). L. casei var. rhamnosus was grown in Lactobacilli MRS broth (Difco) that was sterilized by filtration. All cultures except B. subtilis (Marburg), which was grown with mild aeration, were statically grown at 37 C.

Cell breakage. Overnight cultures were diluted to 37% into their respective media and grown for 100 min at 37 C. Usually, 16 ml of each culture was employed. By using the high inoculum, a relatively large number of cells can be obtained in a small volume. At the end of the 100-min growth period, the cells were harvested by centrifugation (1,000 to 2,000 × g; 0 to 4 C) and washed once with 0.85% (wt/vol) NaCl solution. The pellets, contained in screw-cap tubes (16 by 125 mm), were then each suspended in 1.5 to 2.0 ml of a buffer (0.03 M phosphates, 0.01 M ethylenediaminetetraacetate, 0.02 M 2-mercaptoethanol, pH 6.8) and chilled to 0 C in an ice bath.

Glass beads (1 ml, either 0.11 to 0.12 mm or 0.17 to 0.18 mm, obtained from The Braun Co., Melsungen, Germany, and from the A. H. Thomas Co., Philadelphia, Pa.) were then added, and the starting turbidity at 750 nm was determined on a one to ten dilution (in saline) of each cell suspension. Samples for all dilutions were taken after the beads had settled. The cells plus glass beads were then placed on a mixer (Lab-line Super Mixer, catalog no. 1290, Melrose Park, Ill., or its equivalent) and vortexed for 2 min, with stopping and cooling the cells in an ice bath every 30 s. The temperature rarely rose more than 5 to 10 C. The mixing was done at a maximum setting determined with a stroboscopic light to be 3,360 to 3,380 oscillations per min. After each 30-s interval, the turbidity of each culture was determined as described above, with a Beckman model B spectrophotometer adapted for 8- by 75-mm cuvettes. During the 30-s
vortexing period, it is recommended that the tube be held in the Neoprene head at a slight angle and that the contents of the tube be allowed to settle for a few tenths of a second. This enables all contents to mix thoroughly. Before and after use, the beads were cleaned as described (2).

Cell breakage appears to be independent of the suspending menstruum.

Electron microscopy. Samples of treated cultures were negatively stained and examined in an electron microscope by described methods (7).

Competence development and transformation. WE4 cells were made competent and were transformed by DNA carrying the rifampin marker by described methods (5).

Transforming DNA. WE4 Rif<sup>+</sup> cells were treated as described above, and the centrifuged extract was heated at 56°C for 90 min before being used as a source of transforming DNA. A 0.05-ml volume of extract prepared from 16 ml of culture was added to 1.0 ml of competent cells.

<sup>3</sup>H-labeled DNA. <sup>3</sup>H-labeled DNA was prepared from WE4 cells grown in a semisynthetic medium (4) containing 2.5% (vol/vol) horse serum and titrated thymidine as described (4).

Heat-denatured DNA deoxyribonuclease assay. In general, WE4 <sup>3</sup>H-labeled DNA (0.25 ml, containing about 2,000,000 counts per min per ml) was diluted into 0.6 ml of tris(hydroxymethyl)aminomethane-hydrochloride buffer (1 M, pH 7.8) and heated at 100°C for 10 min. The DNA solution was then chilled rapidly to 0°C in an ice bath. A 0.35-ml volume of water, 0.6 ml of 40 mM MgSO<sub>4</sub>, and 0.6 ml of 140 mM 2-mercaptoethanol were then added and the contents were mixed. A 0.2-ml volume was then added to each reaction tube. This was followed with 0.2 ml of water and 0.1 ml of cell extract. The cell extract consisted of the supernatant fluid derived from cells that were broken as described above and centrifuged (2,000 to 3,000 x g, 0 to 4°C).

The reaction was allowed to proceed at 37°C for 60 min. At 60 min, 0.1 ml of a 40% solution (wt/vol) of trichloroacetic acid and 0.1 ml of a 25% solution (vol/vol) of horse serum were added and the precipitate was allowed to form at 0°C for 10 min. After 10 min, the samples were centrifuged (2,000 to 3,000 x g, 0 to 4°C), and the clear supernatant fluids (0.7 ml) were quantitatively transferred to glass scintillation vials. Aquasol (10 ml; New England Nuclear Corp.) was then added and the acid-soluble radioactivity was determined with a Beckman liquid scintillation counter, model no. LS 355. Controls treated in the same manner but without added enzyme included total radioactivity added (noncentrifuged sample) and background acid-soluble radioactivity added. All samples were counted for 10 min; the two sigma counting error was 2 to 3%.

RESULTS AND DISCUSSION

The types of bacterial cells used in this study, their starting turbidities, and the percentage decrease in their turbidities at 30-s intervals over a 120-s time period after vortexing them with glass beads are shown in Table 1. All cells in the genus Streptococcus were broken. The range was 48 to 75%. In addition to streptococci, cells from other genera were also broken. These included a single culture from the genus Lactobacillus (L. casei var. rhamnosus), a single culture from the genus Staphylococcus (S. aureus, 6538P), a single culture from the genus Bacillus (B. subtilis Marburg), and two cultures from the genus Escherichia (E. coli B and E. coli TAU). The range of breakage was 56 to 92%. In other experiments, E. coli B cultures starting with turbidities of 10 were broken over 85% after 3 min of shaking. A semi-log plot of the turbidity remaining versus the time of vortexing revealed that the decrease followed, for the most part, first-order kinetics. The slow start seen with cultures of FW 229 and K56 reflects long chains of cells. Cell breakage was confirmed by observing negatively stained samples with the electron microscope where ruptured cell walls and fragmented membranes were seen.

In similar experiments, a decrease in cell viability was measured after vortexing cultures of S. aureus (6538P) and S. pyogenes (K56) in the presence of glass beads (Table 2). Cultures of S. aureus (6538P) lost viability at essentially the same rate as they lost turbidity. Cultures of S. pyogenes (K56), however, behaved anomalously. As the turbidity decreased, cell viability first increased, and then decreased. This result is probably due to breaking long chains of cells first (1) and then the cells themselves.

In addition to observing cell breakage and a loss in cell viability, a single enzyme activity was assayed (a deoxyribonuclease that reacts with heat-denatured DNA). All of the cultures listed in Table 1 contained such an enzyme. Essentially all of the heat-denatured DNA added was degraded to acid-soluble material by extracts derived from each culture (data not shown).

Highly active transforming DNA was also obtained in the same manner from WE4 Rif<sup>+</sup> cultures. The extract described in Materials and Methods transformed competent WE4 cells to rifampin resistance with a frequency of 17%. Appropriate controls showed that no live WE4 Rif<sup>+</sup> cells were added.

The results presented in this report introduce a simple procedure for breaking some relatively refractive bacterial cells by vortex-mixing cell suspensions in the presence of glass beads. The convenience and simplicity of the method allows for easy survey of the progress of breakage,
the maintenance of reduced temperatures, and the maintenance of the gas phase above the samples. In a single experiment, air was replaced with nitrogen by flushing the samples, contained in vacutainer tubes (no. 3200 NT; Becton-Dickinson), with nitrogen gas.

Other assets for the procedure are (i) reduced aerosols, since breakage is done in screw-capped tubes, (ii) the glass beads can be sterilized by autoclaving them in situ, and (iii) for large number of cultures, cells can be grown and broken in the same tube. The method should also be applicable to the disruption of yeasts, fungi, and tissue-culture cells.

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**LITERATURE CITED**

1. Hahn, J. J., and R. M. Cole. 1962. Time and concentration relationships in the long-chain reaction of group A streptococci in homologous antiserum and an improved method for evaluation of test results. J. Bacteriol. 83:85–96.

2. Huff, E. H., Oxley, and C. S. Silverman. 1964. Density-gradient patterns of *Staphylococcus aureus* cells and cell walls during growth and mechanical disruption. J. Bacteriol. 88:1155–1162.

3. Neihof, R. A., and W. H. Echols. 1968. Biophysical studies of microbial cell walls. I. The preparation of isolated cell walls. Naval Research Laboratory Report No. 6699, Naval Research Laboratories, Washington, D.C.

4. Parsons, C. L., J. M. Ranhand, C. G. Leonard, A. E. Colon, and R. M. Cole. 1973. Inhibition of transformation in group H streptococci by lysogeny. J. Bacteriol. 113:1217–1222.

**TABLE 1. Breakage of cells from different genera**

| Genera, species, and strain | Starting turbidity, (750 nm) | Percentage decrease in turbidity |
|----------------------------|-------------------------------|---------------------------------|
|                            | 0a                           | 30                             | 60   | 90    | 120   |
| *Streptococcus sanguis* (WE4) | 2.8                          | 25                            | 43    | 43    | 48    |
| *S. sanguis* (FW 229)      | 2.4                          | 3                             | 24    | 35    | 50    |
| *S. pyogenes* (K 56)       | 2.8                          | 3                             | 39    | 50    | 69    |
| *S. mutans* (BHT)          | 2.3                          | 21                           | 38    | 53    | 67    |
| *S. mutans* (AHT)          | 2.2                          | 19                           | 36    | 50    | 69    |
| *S. mutans* (3720)         | 2.5                          | 28                           | 43    | 61    | 74    |
| *S. mutans* (FA-1)         | 1.8                          | 24                           | 37    | 64    | 75    |
| *Lactobacillus casei var. rhamnosus* (Marburg) | 7.7*                       | 20                           | 34    | 48    | 56    |
| *Bacillus subtilis* (Marburg) | 3.7                          | 33                           | 54    | 63    | 78    |
| *Staphylococcus aureus* (6538P) | 2.8                          | 19                           | 43    | 62    | 68    |
| *Escherichia coli* B       | 4.2                          | 52                           | 74    | 80    | 86    |
| *E. coli* TAU              | 6.0                          | 58                           | 74    | 86    | 92    |

* *Seconds.*

* From 8 ml of culture. All others are from 16 ml of culture.

**TABLE 2. Effect of vortexing cultures of *S. aureus* (6538P) and *S. pyogenes* (K56) in the presence of glass beads on their turbidity and viability**

| Culture | Vortexing time (s) | Percentage decrease in turbidity* | Colony counts/ml (× 10⁴) | Percentage increase (+) or decrease (−) (colony counts/ml) |
|---------|-------------------|---------------------------------|--------------------------|----------------------------------------------------------|
| *S. aureus* (6538P) | 0                 | 0                              | 3.4                      | 0                                                        |
|         | 30                | 37                             | 2.2                      | −35                                                       |
|         | 60                | 54                             | 1.6                      | −53                                                       |
|         | 90                | 73                             | 0.6                      | −82                                                       |
|         | 120               | 80                             | 0.3                      | −91                                                       |
| *S. pyogenes* (K56) | 0                 | 0                              | 0.7                      | 0                                                        |
|         | 30                | 20                             | 1.4                      | +100                                                     |
|         | 60                | 37                             | 1.5                      | +115                                                     |
|         | 90                | 59                             | 1.1                      | +57                                                      |
|         | 120               | 66                             | 0.6                      | −14                                                      |

* Cells were grown in brain heart infusion minus horse serum and treated further as described in Materials and Methods. Glass beads (0.11 to 0.12 mm) and cuvettes were sterilized by autoclaving. The suspending menstruum was 0.85% (vol/vol) NaCl.

* Initial turbidities measured at 750 nm were as follows: *S. aureus* (6538P), 2.8; *S. pyogenes* (K56), 2.1.
5. Ranhand, J. M. 1973. Autolytic activity and its association with the development of competence in group H streptococci. J. Bacteriol. 115:607-614.
6. Ranhand, J. M., and R. M. Cole. 1971. Demonstration of transforming deoxyribonucleic acid in an autolysate of a group H Streptococcus. J. Bacteriol. 106:712-713.
7. Ranhand, J. M., C. G. Leonard, and R. M. Cole. 1971. Autolytic activity associated with competent group H streptococci. J. Bacteriol. 106:257-268.
8. Rogers, H. J., and H. R. Perkins. 1968. In C. Long (ed.), Cell walls and membranes, p. 196-206. E. and F. N. Spon LTD, London.
9. Salton, M. R. J. 1964. The bacterial cell wall, p. 42-65. Elsevier Publishing Co., Amsterdam, The Netherlands.