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A Simple and Generally Applicable Procedure for Releasing DNA from Bacterial Cells

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Treatment of *Staphylococcus simulans* biovar *staphylolyticus* cells with acetone before digestion with lysozyme made the cells susceptible to lysis by sodium dodecyl sulfate. This technique was found to be useful for releasing DNA from a wide variety of gram-positive and gram-negative organisms.

The lack of a procedure for release of high-molecular-weight DNA from *Staphylococcus simulans* biovar *staphylolyticus* ("*S. staphylolyticus*" [15]) has hindered molecular genetic studies of exoprotein production by this organism. Lysostaphin is routinely used to release intracellular components from other staphylococcal cells (4, 6, 10), but *S. simulans* biovar *staphylolyticus*, the lysostaphin-producing organism, is quite resistant to this bacteriolytic preparation (5, 13).

Bhaduri and Demchick (1) recently reported a simple and rapid method for disruption of bacteria to release intracellular protein. This procedure involves treatment of the cells with acetone and subsequent extraction of cellular proteins with sodium dodecyl sulfate (SDS). We tested their procedure for its ability to release DNA from *S. simulans* biovar *staphylolyticus*, but very little DNA could be detected. However, the possibility existed that extraction with acetone might increase the susceptibility of the cells to digestion by bacteriolytic enzymes. In fact, *Staphylococcus aureus* cells have been extracted with acetone and then with alcohol before lysis by lysostaphin (14), although the roles of the solvent treatments are unclear. Upon testing we found that acetone-extracted, lysozyme-digested *S. simulans* biovar *staphylolyticus* cells were readily lysed by addition of SDS, thereby releasing DNA.

Small-scale preparations of DNA from *S. simulans* biovar *staphylolyticus* for analytical purposes were made as follows. A 1.0-ml sample of cells from a shaken overnight culture grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) at 37°C was centrifuged in a microcentrifuge (Model 235; Fisher Scientific Co., Pittsburgh, Pa.) at room temperature, and the cells were suspended in 0.5 ml of 50 mM Tris hydrochloride buffer (pH 7.2). The cells were sedimented again, suspended in 0.5 ml of ice-cold acetone, and kept on ice for 5 min. The cells were then resedimented, the acetone was decanted, and residual acetone was removed with a gentle stream of air. The cells were then suspended in 0.25 ml of TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM disodium EDTA). Lysozyme was added to a final concentration of 1 mg/ml, and the mixture was incubated at 37°C for 30 min. Lysis was achieved by the addition of SDS to a final concentration of 1% (wt/vol). NaCl was added to a final concentration of 1 M, and the mixture was kept at −20°C for 1 h. Cellular debris was removed by centrifugation at 4°C for 15 min. A 0.3-ml sample of supernatant was treated at 37°C with RNase A (type I-A; Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 200 μg/ml for 15 min and then with proteinase K (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) at a final concentration of 50 μg/ml for 30 min. The supernatant was then extracted twice with an equal volume of a mixture of chloroform and isoamyl alcohol (24:1, vol/vol) and then with 2 volumes of ether. The DNA was precipitated with 2.5 volumes of 95% ethanol at −20°C overnight and was collected by centrifugation at 4°C for 15 min. The precipitate was dried in a vacuum desiccator and suspended in 25 μl of TE buffer for electrophoretic analysis (9).

For preparative-scale purification of DNA from *S. simulans* biovar *staphylolyticus*, 100 ml of culture was used. All centrifugations were performed with an RC-5B centrifuge with an SS-34 rotor (Ivan Sorvall, Inc., Norwalk, Conn.). Samples were prepared essentially as described above, with the following modifications. After the addition of SDS, the samples were mixed twice for 30 s each with a vortex mixer at full speed, as recommended by Olsen et al. (12). This treatment shears the chromosomal DNA to minimize its precipitation with cellular debris. After the addition of NaCl, the mixture was kept at −20°C overnight and was then centrifuged at 12,000 rpm at 4°C for 45 min. After treatment of the supernatant with RNase A and proteinase K, the chloroform-isoamyl alcohol extractions were performed with 2 volumes per extraction, and the ether extraction was omitted. Centrifugation after the ethanol precipitation was at 12,000 rpm and 4°C for 45 min. After vacuum desiccation, the DNA was suspended in 1 ml of TE buffer and dialyzed against 1 liter of the same buffer at 4°C for 2 h and then against 1 liter of fresh TE buffer at 4°C overnight.

The procedure of Bhaduri and Demchick (1) yielded very little DNA from *S. simulans* biovar *staphylolyticus* (Fig. 1A, lane 2). However, the analytical procedure described above, which involved lysozyme digestion after the acetone extraction, did release a large amount of DNA (Fig. 1A, lane 3). Electrophoretic analysis revealed the presence of chromosomal DNA and at least six discrete bands of extrachromosomal DNA. It was deduced that the intense band was chromosomal DNA by its presence in large amounts in all preparations and its relative position in the gels and because *Eco*RI digestion of a preparation similar to that shown in Fig. 1B, lane 1, produced a smeared ladder of DNA fragments (data not shown). The possible relationships among the bands of plasmid DNA are currently under investigation.

DNA obtained by the preparative procedure from *S. simulans* biovar *staphylolyticus* is shown in Fig. 1B. This procedure yielded chromosomal DNA that was greater than 35.9 kilobases (kb) in size, as indicated by comparison to

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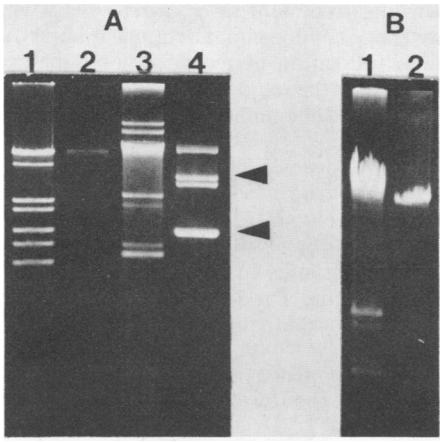

FIG. 1. Gel electrophoresis of DNA from *S. simulans* biovar *staphylolyticus*. DNA preparations were electrophoresed through 1% (A) or 0.6% (B) agarose in Loening E buffer without SDS (7) and were stained with ethidium bromide (9). (A) Analytical procedure. Lanes: 1, linear standards obtained by *Eco*RI digestion of adenovirus 2 DNA (21.3, 4.3, 3.7, 2.7, 2.2, and 1.7 kb) and *Bam*HI digestion of plasmid pSA4 (9.4 kb); 2 and 3, DNA released by treatment with acetone-SDS and acetone-lysozyme-SDS, respectively, and purified by the analytical procedure described in the text; 4, plasmids pSA4 (9.4 kb) and pBR322 (4.4 kb). The arrows indicate supercoiled monomers. (B) Preparative procedure. Lanes: 1, DNA released by acetone-lysozyme-SDS treatment and purified by the preparative procedure described in the text; 2, adenovirus 2 DNA (35.9 kb).

adenovirus 2 DNA. This is of appropriate size for the preparation of random genomic fragments for cloning. As expected, the shearing step in the preparative procedure increased the yield of chromosomal DNA by decreasing its precipitation with cellular debris (12). The increased recovery of chromosomal DNA by this procedure caused a decrease in the relative concentrations of plasmid DNAs to the point that these bands often were only marginally detectable, even on overloaded gels. Furthermore, the excess chromosomal DNA on such overloaded gels sometimes obscured the large-plasmid bands. Finally, the ability to detect the large plasmids may have been even further decreased by their having been sheared to molecules that were not resolved from chromosomal DNA fragments.

We tested the general applicability of the analytical procedure developed for preparation of DNA from *S. simulans* biovar *staphylolyticus*. The strains and growth conditions that were used are given in Table 1. The analytical procedure released DNA from a variety of gram-positive organisms (Fig. 2). This procedure was also found to be effective in releasing DNA from a variety of gram-negative organisms (data not shown). Although many of these gram-negative organisms yield DNA without preliminary acetone extraction, this step may be useful for strains that are ineffectively lysed by procedures that use only lysozyme and a detergent.

To determine whether exposure of cells to acetone altered the native biochemical or biological properties of DNA, susceptibility to restriction endonuclease digestion and the ability to transform were tested. Because the analytical and preparative procedures described above yielded chromosomal fragments that would interfere in restriction endonuclease analysis, plasmid DNA was specifically purified by the procedure of Birnboim (2). Plasmid pBR322 DNA was released from *Escherichia coli* DH1 cells that either were or were not treated with acetone. The electrophoretic patterns of *Eco*RI and *Hin*cII fragments obtained from these preparations by digestion under the conditions recommended by the supplier (Bethesda Research Laboratories) are shown in Fig. 3. The patterns obtained from both preparations were

TABLE 1. Strains and conditions of growth

Organism	Medium[a]	Temperature (°C)	O_2[b]	Source[c]
Staphylococcus simulans biovar *staphylolyticus* NRRL B-2628	TSB	37	+	NRRL
Staphylococcus aureus ATCC 12600	TSB	37	+	ATCC
Staphylococcus epidermidis	TSB	37	+	This laboratory
Streptococcus faecalis ATCC 19433	TSB	37	+	ATCC
Bacillus subtilis ATCC 6051	TSB	37	+	ATCC
Clostridium perfringens	CM	37	−	UA
Lactobacillus plantarum ATCC 8014	MIB	37	(+)	ATCC
Corynebacterium xerosis	TSB	37	+	UA
Mycobacterium smegmatis	TSB	37	+	UA
Arthrobacter sp.	TSB	30	+	UA
Nocardia sp.	TSB	30	+	UA
Escherichia coli	TSB	37	+	This laboratory
Salmonella enteritidis	TSB	37	(+)	UA
Klebsiella pneumoniae ATCC 13883	TSB	37	+	ATCC
Enterobacter aerogenes	TSB	37	+	UA
Proteus mirabilis ATCC 9240	TSB	37	+	ATCC
Serratia marcescens	TSB	30	(+)	UA
Pseudomonas aeruginosa	TSB	37	+	This laboratory
Pseudomonas acidovorans ATCC 15668	TSB	30	+	ATCC
Neisseria subflava ATCC 14799	BHI	37	(+)[d]	ATCC
Rhodospirillum rubrum ATCC 9791	RRB	22	−[e]	ATCC

[a] Abbreviations: TSB, Trypticase soy broth (BBL); CM, cooked meat medium (Difco Laboratories, Detroit, Mich.); MIB, micro inoculum broth (Difco); BHI, brain heart infusion (Difco); RRB, *Rhodospirillum rubrum* broth (0.2% yeast extract [Difco], 0.2% Bacto-Peptone [Difco], 0.05% malate, adjusted to pH 7.0).

[b] +, Aerated by shaking; −, anaerobic; (+), unshaken.

[c] Abbreviations: NRRL, Northern Regional Research Center, Peoria, Ill.; ATCC, American Type Culture Collection, Rockville, Md.; UA, departmental culture collection.

[d] Grown in the presence of 10% CO_2.

[e] Grown photosynthetically.

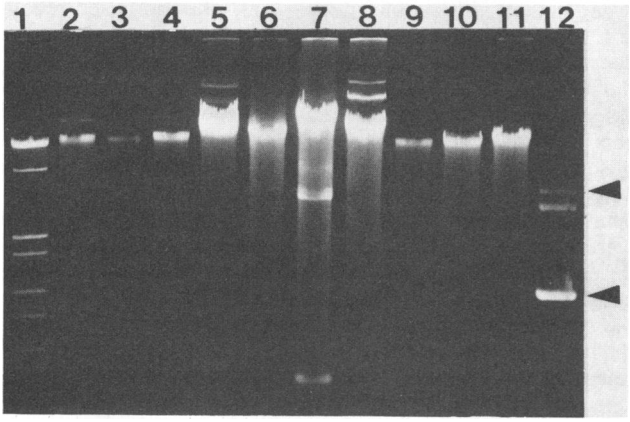

FIG. 2. Gel electrophoresis of DNA from various gram-positive organisms. Preparations were electrophoresed through 1% agarose in Loening E buffer without SDS (7) and were stained with ethidium bromide (9). Lanes: 1, linear standards obtained by *Eco*RI digestion of adenovirus 2 DNA (21.3, 4.3, 3.7, 2.7, 2.2, and 1.7 kb) and *Bam*HI digestion of plasmid pSA4 (9.4 kb); 2 through 11, DNA prepared by the analytical procedure described in the text from *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Bacillus subtilis*, *Clostridium perfringens*, *Lactobacillus plantarum*, *Corynebacterium xerosis*, *Mycobacterium smegmatis*, *Arthrobacter* sp., and *Nocardia* sp., respectively; 12, plasmids pSA4 (9.4 kb) and pBR322 (4.4 kb). The arrows indicate supercoiled monomers.

identical for each enzyme. Similarly obtained preparations of plasmid pUC18 gave identical patterns after *Ava*II digestion (data not shown). In fact, direct treatment of plasmid DNA with acetone did not affect the pattern of fragments produced by either *Eco*RI or *Sau*3A (data not shown). Furthermore, both pBR322 preparations transformed $CaCl_2$-treated (8) *E. coli* MM294 cells with equal efficiency. Others have shown that treatment of *S. aureus* cells with acetone and then with alcohol does not affect plasmid DNA renaturation or its hybridization in reassociation kinetics analyses (11, 14). Therefore, the exposure of cells to acetone does not alter any of these biochemically and biologically relevant properties of DNA.

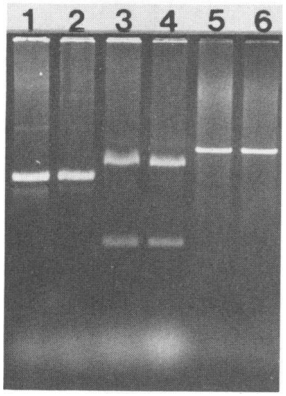

FIG. 3. Gel electrophoresis of restriction endonuclease-digested pBR322 DNA. Samples were electrophoresed through 1% agarose in Loening E buffer without SDS (7) and were stained with ethidium bromide (9). DNA was prepared by the method of Birnboim (2) after acetone treatment (lanes 1, 3, and 5) or without acetone treatment (lanes 2, 4, and 6) of *E. coli* DH1 cells. Lanes: 1 and 2, undigested DNA; 3 and 4, *Hin*cII-digested DNA; 5 and 6, *Eco*RI-digested DNA.

In summary, we described a convenient and rapid method for releasing DNA from a variety of bacteria. Because this technique was developed for the release of DNA from *S. simulans* biovar *staphylolyticus*, the use of different solvents or bacteriolytic enzymes may improve the yield of DNA from other organisms. The DNA may then be purified by a procedure appropriate for the intended application (2, 3, 4).

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