Note

Pretreatment of Cells of *Klebsiella pneumoniae* with 50% (v/v) Dimethylsulfoxide Yields Purified Deoxyribonucleic Acid of Low Polysaccharide Content

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*Klebsiella pneumoniae* is found on plants, insects, animals, and in soil and water. Because it is a heavily encapsulated bacterium, modifications of the fundamental Marmur technique have been required to extract polysaccharide-uncontaminated DNA for genetic and taxonomic study. Capsular polysaccharide has been reported to be removed from DNA preparations by precipitation with 2-methoxyethanol after cell lysis and deproteinization. Cetyltrimethylammonium bromide has also been used to purify free DNA.

An alternative is to remove the capsule before cell lysis, thus minimizing contact with the DNA. In the procedure reported here, single-step pretreatment of cells of *K. pneumoniae* with 50% (v/v) dimethylsulfoxide (DMSO) successfully reduced polysaccharide contamination to negligible levels, as evidenced by spectroscopic data.

**EXPERIMENTAL**

Log-phase cultures of *K. pneumoniae* and *Escherichia coli* K12 were harvested from glucose, mineral salts broth and the cells resuspended in ice-cold PE buffer (0.02 M disodium phosphate, 0.005 M disodium EDTA dihydrate, pH 8.0). Following recentrifugation, 10 g of wet cell paste was dissolved with vortex mixing in 10 ml of PE buffer. To the cell suspension, 10 ml of reagent ACS grade DMSO (Eastman Organic Chemicals, Rochester, N. Y.) was added dropwise with vortex mixing. An exothermic reaction of solvation occurred, bringing the temperature of the mixture to about 30°C. After 10 min mixing, the cells were centrifuged at 4°C, the supernatant decanted, and the cell pellet twice resuspended in 10 ml PE buffer and centrifuged to remove residual DMSO. Smears from the cell pellet before and after DMSO extraction were stained for capsule and examined microscopically.

DNA was then extracted using Goodgal’s modification of the Marmur technique, with the following points. Isopropanol and sodium perchlorate were used as originally described by Marmur in place of 95% ethanol and sodium chloride used by Goodgal. After each deproteinization, isopropanol-precipitated, spooled DNA was redissolved in 0.1 x SSC (0.015 M NaCl, 0.0015 M trisodium citrate in distilled water, pH 7.0), as first suggested by Mandel and coworkers, in place of the sodium-EDTA reagent used by Goodgal. After the initial extraction and RNAase treatment (steps 6 through 11 of Goodgal), deproteinization (steps 12 through 14 of Goodgal) was repeated three times. Step 15 of Goodgal was omitted. Lastly, to assure that the final solution of purified DNA would be at the standard ionic strength required for spectroscopic analysis, precipitated and spooled DNA (step 13 of Goodgal) was then dipped in a 95% ethanol bath to remove excess salt, rinsed with 0.1 x SSC, blotted to remove excess fluid, and redissolved in 0.1 x SSC.

Analysis of the purified DNA was performed using a Gilford Model 2527 thermoprogammable UV spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio). The thermal denaturation profile was started after a baseline was established at 60°C, and the temperature advanced at 0.5°C min⁻¹ to 90°C while monitoring hyperchromicity at 260 nm. $T_m$, the temperature at the half-point in the melting curve, was determined graphically from the chart, and percent guanine plus cytosine, % (G + C), was calculated with the formula,

$$\% (G+C) = 100 \left[ \frac{T_m}{50.2} - 0.99 \right]$$

appropriate for the ionic strength in 0.1 x SSC. Optical density of undenatured DNA at 230, 260, and 280 nm was also recorded. Renaturation upon cooling was observed, but not studied. The DMSO supernatant was not assayed for organic solutes.

**RESULTS**

The results are presented in Table I. Optical density ratios, $A_{260}/A_{230}$ and $A_{260}/A_{280}$, of higher than 2.30 and 1.95, respectively, indicate negligible polysaccharide and protein contents of the purified DNA. Hyperchromic shifts at 260 nm (not shown) of about 35% demonstrate a good proportion of double-stranded DNA in the extracts.

Two reference strains, *E. coli* K12 strain ATCC 23724 and *K. pneumoniae* strain ATCC 13883, were studied. For the unencapsulated bacterium, *E. coli* K12, pretreatment with DMSO resulted in a slight increase in $T_m$ from 75.7°C to 76.0°C. % (G + C) was calculated to be 52.3%. For comparison, a published value, without extraction of polysaccharides, is 51.7%. For *K. pneumoniae* ATCC 13883, pretreatment with DMSO resulted in a marked increase in the $T_m$ from 77.3°C to 78.8°C. % (G + C) was calculated to be 58.0%. A published value, using 2-methoxyethanol to remove polysaccharide, is 57.8%.

An environmental isolate, *K. pneumoniae* strain 10 KP, and its acapsulate mutant, strain 30KP (author's collection), were also compared. Whereas for the acapsulate mutant, $A_{260}/A_{230}$ was stable at about 2.5, for the mucoid...
TABLE I. SPECTROPHOTOMETRIC DATA AND THERMAL DENATURATION ANALYSIS OF DNA EXTRACTED FROM DMSO PRETREATED (∆) AND UNTREATED (▽) CELLS OF Klebsiella pneumoniae and Escherichia coli K12

<table>
<thead>
<tr>
<th>Strain</th>
<th>DMSOa</th>
<th>$A_{260}/A_{230}$b</th>
<th>$A_{260}/A_{280}$c</th>
<th>$T_m$d</th>
<th>% (G+C)e</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli K12 ATCC 23724</td>
<td>∆</td>
<td>2.54</td>
<td>2.02</td>
<td>76.0</td>
<td>52.3</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>2.44</td>
<td>1.99</td>
<td>75.7</td>
<td>51.8</td>
</tr>
<tr>
<td>K. pneumoniae ATCC 13883</td>
<td>∆</td>
<td>2.42</td>
<td>1.97</td>
<td>78.8</td>
<td>58.0</td>
</tr>
<tr>
<td>V</td>
<td></td>
<td>1.75</td>
<td>1.85</td>
<td>77.3</td>
<td>55.4</td>
</tr>
<tr>
<td>K. pneumoniae 10KPf</td>
<td>∆</td>
<td>2.48</td>
<td>2.01</td>
<td>79.2</td>
<td>58.7</td>
</tr>
<tr>
<td>(encapsulated parent)</td>
<td>V</td>
<td>1.71</td>
<td>1.84</td>
<td>77.1</td>
<td>54.6</td>
</tr>
<tr>
<td>K. pneumoniae 30KPf</td>
<td>∆</td>
<td>2.52</td>
<td>2.02</td>
<td>79.3</td>
<td>58.8</td>
</tr>
<tr>
<td>(acapsulate mutant of 10KP)</td>
<td>V</td>
<td>2.48</td>
<td>1.99</td>
<td>79.0</td>
<td>58.2</td>
</tr>
</tbody>
</table>

a DMSO pretreated (△), DMSO untreated (▽), as described in text.
b Optical density ratio at 260 and 230 nm.
c Optical density ratio at 260 and 280 nm.
d Temperature at half-point of thermal melting hyperchromic shift at 260 nm. Determined graphically from chart recorder.
e Percent guanine plus cytosine, % (G+C), calculated from $T_m$.
f Strain lyophiles in author’s collection of Klebsiella pneumoniae.

The results indicate that DMSO pretreatment yields purified DNA with negligible polysaccharide contamination, as evidenced by spectroscopic data. The results of Mandel and coworkers6) show that DNA may be considered physico-chemically pure when $A_{260}/A_{230}$ is 2.2 to 2.5 and $A_{260}/A_{280}$ is 1.94 to 1.96. Graves10) studied the interaction of polysaccharides with DNA over the whole UV spectrum and showed a definite flattening of the hyperchromicity of DNA at 260 nm with marked decreases in the $A_{260}/A_{230}$ and $A_{260}/A_{280}$ ratios in the presence of polysaccharides. Marmur11) was the first to describe the difficulty which the polysaccharide capsule of K. pneumoniae caused in extraction of DNA. Consequently, various modifications of his procedure have been proposed.2,3) In the method reported here, pretreatment of heavily encapsulated cells of K. pneumoniae with DMSO produced clean DNA extractions, and microscopic examination of the cells for capsule showed that after DMSO extraction, the capsule had vanished.

This simple method for removal of capsule may have broad applicability. Highly purified DNA is essential for hybridization and biotechnology studies. Also, it may be that use of DMSO for industrial harvesting of capsular materials will prove successful. It would be of interest to know whether bacteria treated in this manner retain viability for recycling in continuous fermentation processes.

The procedure described here is based on an earlier report by Adams11) concerning extraction of capsular polysaccharides with DMSO. Use of an EDTA buffer system was prompted by studies of Leive.12)

REFERENCES