

# Characterization of a Restriction Endonuclease, *PhaI*, from *Pasteurella haemolytica* Serotype A1 and Protection of Heterologous DNA by a Cloned *PhaI* Methyltransferase Gene

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Received 3 December 1993/Accepted 3 April 1994

*Pasteurella haemolytica* is the leading cause of economic loss to the beef cattle industry in the United States and an important etiologic agent worldwide. Study of *P. haemolytica* is hindered by researchers' inability to genetically manipulate the organism. A new restriction endonuclease, *PhaI*, an isoschizomer of *SfaNI* (R. J. Roberts, *Methods Enzymol.* 65:19–36, 1980), was isolated from *P. haemolytica* serotype 1, strain NADC-D60, obtained from pneumonic bovine lung. *PhaI* recognizes the 5-base nonpalindromic sequences 5'-GCATC-3' and 5'-GATGC-3'. Cleavage occurs 5 bases 3' from the former recognition site and 9 bases 5' from the latter recognition site. A gene encoding a methyltransferase which protects against *PhaI* cleavage was cloned from *P. haemolytica* NADC-D60 into *Escherichia coli*. Whereas unmethylated plasmid DNA containing a *P. haemolytica* origin of replication was unable to transform *P. haemolytica* when introduced by electroporation, the same plasmid DNA obtained from *E. coli* which contained a cloned *PhaI* methyltransferase gene could do so. The data indicate that *PhaI* is an effective barrier to the introduction and establishment of exogenous DNA in *P. haemolytica*.

*Pasteurella haemolytica* biotype A, serotype 1, is the most common etiologic agent responsible for pneumonic pasteurellosis in domestic cattle (8). The organism is prevalent in the upper respiratory tract and palatine tonsils of healthy cattle; the latter site has been found to support infection for long periods of time (8, 9). Under stressful conditions or with a viral respiratory tract infection, *P. haemolytica* can rapidly colonize the upper respiratory passages to become the predominant flora, whereupon it may be inhaled and result in pneumonic pasteurellosis (8). The mechanism of upper respiratory tract colonization and the pathogenesis of pneumonia, however, are still poorly understood (11). Interest in elucidation of the molecular mechanisms of pathogenesis and development of improved vaccine strains has led several researchers to investigate genetic manipulation of *P. haemolytica*.

Shuttle vectors or directed mutants might incorporate portions of plasmids known to exist in the organism. Serotype 1 *P. haemolytica* commonly carries a single 4.2-kb ROB-1  $\beta$ -lactamase-encoding plasmid. A second 4.2-kb plasmid is also common and, if present, is always found together with the 4.2-kb  $\beta$ -lactamase plasmid in field isolates (3, 4). The second plasmid has been sequenced and found to contain genes for sulfonamide and streptomycin resistance (6). In addition to characterization of plasmids in *P. haemolytica*, several genes from this bacterium have been cloned and sequenced, including the gene cluster for leukotoxin, a possible virulence factor which has received considerable attention (12, 13, 15, 16, 23).

Through the use of transposon mutagenesis or site-directed mutagenesis, the role played by potential virulence factors may be assessed and improved vaccines may be designed. Efforts to

introduce foreign DNA into *P. haemolytica* serotype 1, however, have not been particularly successful (2, 6, 7, 10). Whereas plasmid DNA obtained from *P. haemolytica* serotype 1 can be reintroduced into *P. haemolytica* by electroporation without difficulty, *P. haemolytica* plasmid DNA isolated from an *Escherichia coli* host does not transform *P. haemolytica* under similar conditions. Means to establish foreign DNA in *P. haemolytica* serotype 1 would allow researchers to design improved studies of pathogenetic mechanisms and potentially improved vaccines.

The possibility that a restriction barrier prevents establishment of foreign DNA in *P. haemolytica* led us to undertake the present study. This study was designed to identify restriction endonuclease activity in *P. haemolytica* serotype 1 and to characterize the enzyme recovered. In addition, we sought to determine the extent to which specific methylation of foreign plasmid DNA by cloned methyltransferase might enhance transformation of *P. haemolytica* serotype 1.

## MATERIALS AND METHODS

**Bacterium, growth, and crude extract.** For each chromatographic run, *P. haemolytica* serotype 1, strain NADC-D60, was grown for 16 h on four Columbia blood agar base plates (100-ml total volume; Difco, Detroit, Mich.) without supplemental blood. The cells were harvested in TE (10 mM Tris, 1 mM EDTA, pH 8.0), pelleted by centrifugation at 16,000  $\times$  g for 5 min at 4°C, and washed once in TE. The washed pellet was resuspended in 1.5 ml of chromatography running buffer (20 mM sodium phosphate, 10 mM 2-mercaptoethanol, pH 7.5; 4°C) and placed on ice. The bacterial cells were disrupted by sonication for 2 min in 15-s bursts. Debris and unbroken cells were removed by centrifugation at 16,000  $\times$  g for 10 min, and the supernatant was filtered through a 0.45- $\mu$ m-pore-size membrane (Millex-HA; Millipore Corp., Bedford, Mass.). No

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further treatment of the crude extract was performed prior to chromatography.

**Chromatographic separation of proteins.** All chromatographic procedures were performed at room temperature. Prepacked heparin-Sepharose columns (Econo-pac heparin columns; Bio-Rad, Richmond, Calif.) were equilibrated as recommended by the manufacturer. A flow rate of 0.5 ml/min was used for separation, using a gradient high-performance liquid chromatography system (Beckman Instruments, Inc., Fullerton, Calif.). One milliliter of crude extract was injected, and 10 ml of running buffer was used to wash the column. A linear gradient from 0 to 0.5 M NaCl in 60 ml of running buffer was used to elute proteins. The column was washed with 2 M NaCl in running buffer at 2.0 ml/min as recommended by the manufacturer and then reequilibrated to initial conditions of 0 M NaCl in running buffer prior to additional runs. Fractions (1.0 ml) were stored on ice prior to activity assay and then frozen at  $-20^{\circ}\text{C}$ .

**Assay for restriction endonuclease activity.** Aliquots (5  $\mu\text{l}$ ) of the chromatographic fractions were incubated with 1  $\mu\text{l}$  of 12 mM  $\text{MgCl}_2$  and 0.5  $\mu\text{l}$  of unmethylated bacteriophage lambda DNA (0.5  $\mu\text{g}/\mu\text{l}$ ; New England Biolabs, Beverly, Mass.) at  $37^{\circ}\text{C}$  for 2 h. After addition of tracking dye and electrophoresis on a 1% agarose gel in Tris-borate-EDTA buffer, the banding patterns were visualized by ethidium bromide staining and UV illumination. The active fractions were pooled (6 ml), concentrated 10-fold on 20,000-molecular-weight-cutoff ultrafilters, and brought to final concentrations of 150 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 0.25  $\mu\text{g}$  of bovine serum albumin per ml, and 50:50 (vol/vol) glycerol, pH 7.5, for storage at  $-20^{\circ}\text{C}$ . The concentrated preparation was designated *PhaI*.

**Determination of the recognition sites for *PhaI*.** The recognition sequence was identified by digestion of pBluescript (Stratagene, La Jolla, Calif.). Double digestion with *PhaI* and either *XhoI* or *SacI*, which cut at opposite ends of the polylinker, mapped two of the four *PhaI* sites in pBluescript. Additional double digestions with *AvaII*, *BglII*, *DraI*, *PvuI*, and *ScaI* were used to map the remaining two *PhaI* sites. Further confirmation was made with *PhaI* digests of  $\phi\text{X174}$  and pUC19 DNA and by sequencing pBluescript *PhaI* fragments filled in and cloned into pBluescript. Single-stranded  $\phi\text{X174}$  DNA was digested to determine if *PhaI* has activity on this substrate.

**Determination of the cleavage sites for *PhaI*.** The cleavage site was identified by digestion of a primed-synthesis reaction on pBluescript derivatives (5). An oligonucleotide containing the *PhaI* site was annealed and ligated with *SmaI*-cleaved pBluescript SK<sup>+</sup> and SK<sup>-</sup> DNA. Single-stranded DNA containing each orientation was selected and used for the template. Four standard dideoxy DNA sequencing reactions were performed for each template with an appropriate primer. Additional reactions containing no dideoxy terminator were extended through the *PhaI* site with the Klenow fragment of DNA polymerase I by using <sup>32</sup>P-end-labelled primer with both templates. The extension reaction was stopped by phenol-chloroform extraction followed by ethanol precipitation. *PhaI* or *SfaNI* (New England Biolabs) was added to the additional reactions and allowed to digest the DNA for 2 min. The reaction was stopped by addition of gel loading buffer and heating to  $80^{\circ}\text{C}$  for 3 min.

**Cosmid library construction.** High-molecular-weight DNA for cosmid cloning was prepared by the large-scale DNA isolation method described elsewhere for gram-negative bacteria (1). Approximately 100  $\mu\text{g}$  of *P. haemolytica* NADC-D60 genomic DNA was digested with 100 U of *ApoI* in New England Biolabs buffer 3 at  $50^{\circ}\text{C}$  for 10 min. Following

digestion, the DNA was phenol-chloroform extracted and ethanol precipitated. The DNA was resuspended in 100  $\mu\text{l}$  of TE and layered onto a linear gradient of 10 to 40% sucrose (Schwartz-Mann Ultrapure) in 10 mM Tris HCl-1 mM EDTA-100 mM NaCl, pH 8.0. After centrifugation in an SW40 rotor (Beckman Instruments) at 20,000 rpm for 20 h, gradient fractions were collected and restriction fragments approximately 30 kb in length were ligated into *EcoRI*-digested calf alkaline phosphatase-treated cosmid vector pLAFRX (1). A standard ligation mixture contained 1  $\mu\text{g}$  of vector, 3  $\mu\text{g}$  of *P. haemolytica* DNA, and 5 Weiss units of T4 ligase in a volume of 10  $\mu\text{l}$ . The ligation mixture was incubated at  $10^{\circ}\text{C}$  for 16 h. The DNA was packaged by using Promega packaging extract (Promega, Madison, Wis.) according to the manufacturer's recommendations. *E. coli* HB101 transduced with the recombinant cosmid library was plated on  $2\times$  TY plates (1) containing 10  $\mu\text{g}$  of tetracycline per ml. Cloning efficiencies were approximately  $10^4$  recombinant colonies per  $\mu\text{g}$  of genomic DNA.

**Cloning of the *PhaI* endonuclease and methyltransferase gene.** Approximately 1  $\mu\text{g}$  of the recombinant *P. haemolytica* cosmid library was digested with *PhaI* restriction enzyme. The digested DNA was phenol-chloroform-isoamyl alcohol extracted, ethanol precipitated, and resuspended in TE buffer. The DNA was electroporated into *E. coli* AP1-200-9 (18), and the cells were plated on Luria-Bertani broth plates containing 20  $\mu\text{g}$  of tetracycline per ml and 35  $\mu\text{g}$  of 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) per ml. The AP1-200-9 strain of *E. coli* was designed by Piekarowicz et al. to give color selection for functional DNA-modifying genes (18). Briefly, AP1-200-9 contains temperature-sensitive Mrr and Mcr systems which induce damage to modified DNA at nonpermissive temperatures. The strain also contains a *lacZ* gene fused to a damage-inducible SOS locus which allows color selection. In this study, the transformed AP1-200-9 cells were incubated at  $42^{\circ}\text{C}$  for 18 h and transferred to  $30^{\circ}\text{C}$  for 4 h. The cells were moved again to  $42^{\circ}\text{C}$ , and blue colonies, indicating the presence of a cloned methyltransferase gene, were isolated and analyzed. The colonies were screened for restriction endonuclease activity by the technique of Schleif (21). Double-stranded DNA minipreparations isolated from restriction endonuclease-positive colonies were analyzed for resistance to digestion by *PhaI*. Recombinant colonies resistant to *PhaI* digestion were presumed to contain a *PhaI* methyltransferase gene. Cosmid DNA from these cells was electroporated into *E. coli* DH10B (Bethesda Research Laboratories, Gaithersburg, Md.), and the cells were plated on Luria-Bertani broth plates containing 20  $\mu\text{g}$  of tetracycline per ml. The transformants containing the *PhaI* methyltransferase gene were designated *E. coli PhaIMtase*.

**Construction and methylation of hybrid shuttle vector.** The following hybrid DNA construct was generated during attempts to introduce site-directed mutations into *P. haemolytica*. The *aroA* gene of *P. haemolytica*, contained on a *HindIII*-*AccI* fragment of genomic DNA from strain NADC-D60, was ligated into the *HindIII*-*AccI* site of pBluescript (25). A 700-bp fragment was excised from the coding region of the *aroA* gene by double digestion with *NdeI* and *StyI*. Following digestion, the fragment ends were made blunt by treatment with the Klenow fragment of *E. coli* polymerase I and deoxynucleoside triphosphates. The deleted plasmid was excised from a 1% agarose gel and electroeluted. The eluted DNA, designated pPh $\Delta$ aroA2, was phenol-chloroform extracted and ethanol precipitated. The fragment was resuspended in TE buffer and ligated with the  $\text{Cm}^r$  gene isolated from pBR325. The  $\text{Cm}^r$  gene was excised from pBR325 by double digestion

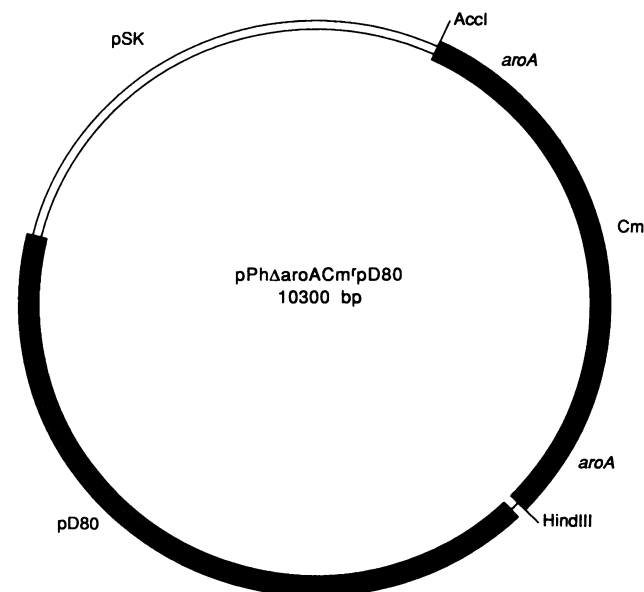


FIG. 1. Physical map of hybrid plasmid pPh $\Delta$ aroACm<sup>r</sup>pD80. A HindIII-AccI fragment of *P. haemolytica* which contains *aroA* was cloned into the HindIII-AccI sites of pBluescript (Stratagene). The *aroA* gene was inactivated by removing 700 bp from *aroA* and blunt ligation of Cm<sup>r</sup>, resulting in pPh $\Delta$ aroACm<sup>r</sup>. A *P. haemolytica* plasmid origin of replication was added by ligating ScaI-digested pD80 into the SmaI site of pBluescript, resulting in the hybrid construct which contains intact Amp<sup>r</sup> genes both from pBluescript and from pD80.

with *AatII* and *ClaI*, and the fragment was made blunt and purified by the above-described methods. The Cm<sup>r</sup> fragment ligated with pPh $\Delta$ aroA2 was given the designation pPh $\Delta$ aroACm<sup>r</sup> (Fig. 1). Transformation of *E. coli* DH10B with pPh $\Delta$ aroACm<sup>r</sup> conferred Cm<sup>r</sup> to the bacterium. The hybrid plasmid pPh $\Delta$ aroACm<sup>r</sup>pD80 was constructed by ligating SmaI-digested pPh $\Delta$ aroACm<sup>r</sup> with ScaI-digested pD80 (4.2-kb Amp<sup>r</sup> plasmid from *P. haemolytica* serotype 1, strain NADC-D80). The resultant hybrid plasmid, approximately 11 kb in size, contained ColE1 and *P. haemolytica ori*, Amp<sup>r</sup>, and Cm<sup>r</sup> (Fig. 1). For methylation, the hybrid plasmid was electroporated into *E. coli* DH10B and *PhaIMtase*. Plasmid DNA was isolated and purified by CsCl gradient centrifugation. Plasmid recovered from *E. coli PhaIMtase* was electroporated into *P. haemolytica* NADC-D60. Plasmid DNA was reisolated from an ampicillin-resistant *P. haemolytica* transformant by the above-described procedures.

#### Transformation of *P. haemolytica* with hybrid plasmid DNA.

*P. haemolytica* NADC-D60 was grown to late logarithmic phase in 250 ml of Columbia broth (Difco) for 3 h at 37°C with shaking. The bacteria were centrifuged at 5,000  $\times$  *g* for 15 min, and the pellet was resuspended in 272 mM sucrose at 0°C. The bacteria were washed four times in 272 mM sucrose with 5 min of centrifugation at 16,000  $\times$  *g* and finally suspended at 50:50 (vol/vol) packed bacteria–272 mM sucrose on ice. Competent bacteria (100  $\mu$ l) were mixed with 1  $\mu$ g of hybrid plasmid DNA from the three sources (*E. coli* DH10B, *E. coli PhaIMtase*, and *P. haemolytica* NADC-D60) in three separate 0.1-cm electroporation cuvettes (Bio-Rad), and there was a fourth no-DNA control. The cells were quickly electroporated after addition of DNA (Gene pulser; Bio-Rad) at 15,000 V/cm, 800  $\Omega$ , and 25  $\mu$ F with resultant time constants ranging from 7.8 to 8.9 ms. Columbia broth (1 ml, 0°C) was immediately added to the

electroporated cells, and the suspension was kept on ice for approximately 10 min. The electroporated cells were allowed to recover at 37°C with gentle shaking for 1 h. One milliliter of broth containing 20  $\mu$ g of ampicillin was added to bring the final ampicillin concentration to 10  $\mu$ g/ml, and the cells were incubated for an additional hour at 37°C with shaking. Tenfold dilutions were plated in duplicate onto blood agar plates containing 5% bovine blood and 10  $\mu$ g of ampicillin per ml. Undiluted cells electroporated with hybrid plasmid obtained from *E. coli PhaIMtase* were plated in duplicate on blood agar plates containing 2  $\mu$ g of chloramphenicol per ml after the first hour of recovery. Colonies were enumerated after overnight incubation at 37°C, and representative colonies were checked for plasmid content.

## RESULTS AND DISCUSSION

Under our experimental conditions, endonuclease activity was eluted from heparin-Sepharose columns by 275 to 325 mM NaCl. A single pass through these columns was sufficient to allow identification of both the DNA recognition specificity and cleavage site. Approximately 5,000 U of *PhaI* per g of wet cells were recovered. Digestion of pBluescript with *PhaI* resulted in four fragments with approximate sizes of 1,476, 1,057, 252, and 184 bp. Double digestion with *PhaI* and either *XhoI* or *SacI* mapped two *PhaI* sites, one at approximately nucleotide 1245 and another at nucleotide 2735 of pBluescript. Additional double digestions with *PhaI* and each of *AvaII*, *BglII*, *DraI*, *PvuI*, and *ScaI* mapped the remaining two *PhaI* sites at approximately nucleotides 2300 and 2490, consistent with the sequences 5'-GATGC-3' and 5'-GCATC-3'. Digests of  $\phi$ X174 and pUC19 DNA and sequencing of pBluescript *PhaI* fragments confirmed the recognition sites (data not shown). These data show that *PhaI* is an isoschizomer of *SfaNI*, a type II enzyme isolated from *Streptococcus faecalis* (20). The type II restriction enzymes, like the more common type II restriction enzymes, recognize specific sequences and cleave at predetermined sites. Type II enzymes, however, do not recognize palindromic sequences nor cleave internally to the recognition sequence (24). Double digests of pBluescript with *PhaI* and *SfaNI* resulted in fragments indistinguishable from those using either enzyme alone. *PhaI* did not cleave single-stranded DNA (data not shown). In contrast to *SfaNI*, optimal conditions for *PhaI* digestion required NaCl or KCl concentrations below 50 mM; >50% reduction in activity was observed at the 100 mM NaCl concentration recommended for *SfaNI*. The cleavage site was found to be identical to that of *SfaNI*, demonstrating that the two are true isoschizomers. DNA containing the recognition sequence 5'-GATGC-3' cut 9 nucleotides 5' to the end of the recognition site with both *PhaI* and *SfaNI* (Fig. 2, lanes 1 and 2), and DNA containing the recognition sequence 5'-GCATC-3' cut 5 nucleotides 3' to the end of the recognition site with both *PhaI* and *SfaNI* (Fig. 2, lanes 3 and 4):



After digestion with *PhaI* and transformation of the AP1-200-9 strain of *E. coli*, 15 cosmid clones of *P. haemolytica* genomic DNA were tested for endonuclease activity. The nine clones which were endonuclease positive were tested for *PhaI* methyltransferase activity. All nine expressed methyltransferase activity in addition to endonuclease activity, as evidenced by resistance to digestion by *PhaI* of genomic DNA recovered from transformed *E. coli* (Fig. 3). The selective recovery of clones containing functional methyltransferase was due to previous digestion of the cosmid library with *PhaI* prior

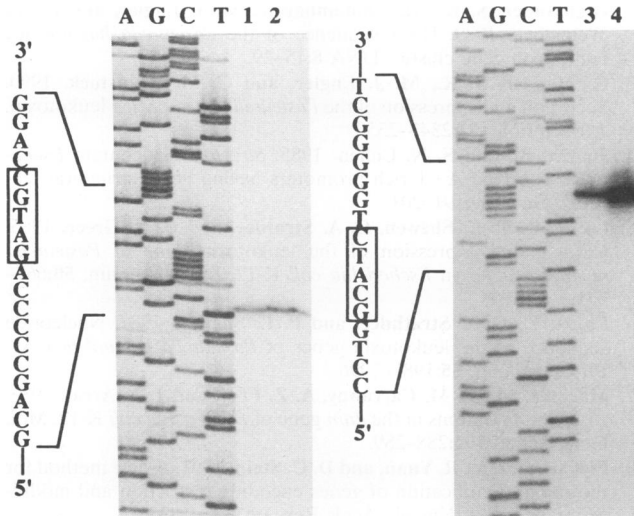


FIG. 2. Determination of *PhaI* cleavage positions alongside that of *SfaNI*. Lanes 1 and 3, cut with *PhaI*; lanes 2 and 4, cut with *SfaNI*. The cleavage products of *PhaI* and *SfaNI* migrated 0.5 bp faster than the corresponding sequence bands because the labelled primer for extension had a 5' phosphate whereas the primer for sequencing did not (14).

to transformation of *E. coli*. Recovery of clones containing both *PhaI* endonuclease and methyltransferase activities is not surprising, since restriction and modification enzymes have previously been shown to be closely linked (the proximity of such genes has obvious implications for gene inheritance and for the survival of the organism) (26).

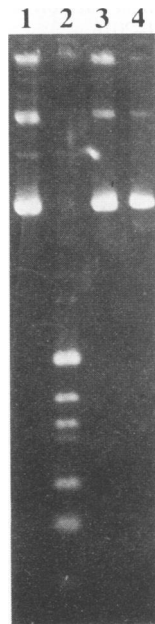


FIG. 3. Protection against *PhaI* digestion by cloned *PhaI* methyltransferase. Lanes 1 and 2, plasmid pPh $\Delta$ aroACm'pD80 from *E. coli* DH10B incubated without and with *PhaI*, respectively; lanes 3 and 4, plasmid pPh $\Delta$ aroACm'pD80 from *E. coli* *PhaIMtase* incubated without and with *PhaI*, respectively.

TABLE 1. Transformation efficiency of *P. haemolytica* NADC-D60 with hybrid plasmid pPh $\Delta$ aroACm'pD80 purified from various sources<sup>a</sup>

Source of DNA <sup>b</sup>	Amp <sup>r</sup> transformants <sup>c</sup> (CFU/ $\mu$ g of DNA)	Cm <sup>r</sup> transformants <sup>d</sup> (CFU/ $\mu$ g of DNA)
<i>E. coli</i> DH10B	0	ND
<i>E. coli</i> <i>PhaIMtase</i>	$1 \times 10^3$	5
<i>E. coli</i> GM2163	$5 \times 10^2$	ND
<i>P. haemolytica</i> NADC-D60	$1 \times 10^5$	ND

<sup>a</sup> One microgram of DNA was introduced by electroporation using the same competent cell preparation.

<sup>b</sup> Purified by CsCl-ethidium bromide gradient centrifugation.

<sup>c</sup> Colonies on plates containing 10  $\mu$ g of ampicillin per ml; cells were recovered 2 h prior to plating.

<sup>d</sup> Colonies on plates containing 2  $\mu$ g of chloramphenicol per ml; cells were recovered 1 h prior to plating. ND, not done.

Hybrid plasmid pPh $\Delta$ aroACm'pD80 passed through *E. coli* containing *PhaI* methylase in a cosmid vector was able to transform *P. haemolytica* serotype 1. Plasmid pPh $\Delta$ aroACm', probably because it contains only ColE1 *ori*, was unable to transform *P. haemolytica* serotype 1. The hybrid plasmid was stably maintained through multiple passages under selective pressure. Whereas DNA not exposed to *PhaI* methylase did not transform *P. haemolytica* in this experiment, DNA modified by *PhaI* methyltransferase yielded  $10^3$  transformants per  $\mu$ g of plasmid (Table 1). Plasmid DNA passed through *P. haemolytica* yielded  $10^5$  transformants per  $\mu$ g of plasmid. Plating efficiency was approximately 100-fold higher with ampicillin selection than with chloramphenicol selection. Fewer than  $10^1$  transformants per  $\mu$ g of plasmid were recovered under chloramphenicol selection using DNA passed through *E. coli* containing *PhaI* methyltransferase. All transformants recovered, however, were resistant to both ampicillin and chloramphenicol upon passage.

While DNA modification by the *PhaI* methyltransferase gene conferred a considerable improvement in transformation efficiency, DNA modification by *P. haemolytica* itself apparently is better still. It is possible that systems analogous to *E. coli* Mcr and Mrr are active in *P. haemolytica*, which might account for the lower transformation efficiency of a hybrid plasmid isolated from *E. coli* *PhaIMtase* compared with that of a plasmid isolated from *P. haemolytica*. This possibility was investigated by passage of pPh $\Delta$ aroACm'pD80 through *E. coli* GM2163 previously transformed with the recombinant cosmid containing *PhaI* methyltransferase (19). *E. coli* DH10B carries *dam*<sup>+</sup>, so hybrid plasmid obtained from *E. coli* *PhaIMtase* would contain certain methyladenines in addition to modification at *PhaI* sites. Since strain GM2163 is a *dam* and *dcm* mutant, the resultant DNA would be modified only at *PhaI* sites and not contain foreign methylation (17). Efficiency of transformation with the latter hybrid plasmid DNA, however, was not substantially different from that with DNA obtained from *PhaIMtase* (Table 1). It is possible a second restriction system, not readily detectable in cell extracts, is active in *P. haemolytica* serotype 1. Genes in *Neisseria gonorrhoeae* MS11 which encode restriction enzymes which are expressed at levels too low to detect biochemically have been described elsewhere (22).

This experiment demonstrates that the restriction modification system of *PhaI* plays an important role in the difficulties that researchers have encountered in their attempts to introduce exogenous DNA into *P. haemolytica* serotype 1. Protection against *PhaI* activity may allow genetic manipulation of

this organism, which could lead to dramatic improvements in our understanding of pathogenesis and control of pneumonic pasteurellosis in cattle.

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