Analysis of partial 16S rRNA nucleotide sequences of
Chlamydia pecorum and C. psittaci

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Abstract

Partial 16S nucleotide sequences of Chlamydia psittaci isolates S26/3 (aboration), P94/1 (pigeon) and Chlamydia pecorum
isolates W73 (enteric) and E58 (encephalomyelitis) were determined. Analysis of these data indicates very high levels of
interspecies sequence conservation, with C. psittaci being more closely related to C. pecorum than to C. pneumoniae or C.
trachomatis. Restriction enzyme analysis of nucleotide sequences indicated that BsuI can be used to clearly distinguish C.
psittaci and C. pecorum isolates. Psittacine and non-psittacine (pigeon) avian isolates of C. psittaci were distinguished using
MacI.

Keywords: Chlamydia species; Restriction enzyme; Interspecies relationship

1. Introduction

The genus Chlamydia comprises four species, Chlamydia psittaci, Chlamydia trachomatis [1], Chlamydia pneumoniae [2] and Chlamydia pecorum [3]. The most important diseases caused by chlamydiae
in humans are trachoma and urogenital infections (C. trachomatis), respiratory infections due to C. pneumoniae and psittacosis caused by C. psittaci [4]. In animals, C. psittaci and C. pecorum give rise
to a wide variety of diseases including abortion, pneumonia, Enteritis, polyarthritis, encephalomyelitis and conjunctivitis [5].

Analysis of C. trachomatis, C. psittaci and C. pneumoniae 16S ribosomal RNA (rRNA) sequences has indicated high levels of interspecies homology, with C. pneumoniae being more closely related to C. psittaci than to C. trachomatis [6]. Using these
data, specific 16S rRNA primers were designed to allow differentiation of C. psittaci, C. pneumoniae and C. trachomatis using the polymerase chain reaction (PCR) [7,8]. Minor differences in the migration patterns of DNA generated by restriction en-
zyme (RE) digestion of PCR products amplified from the 16S rRNA gene allowed C. psittaci abortion strains to be distinguished from C. pecorum strains [9,10].

In the present study, a region of the 16S rRNA gene of C. pecorum isolates W73 (ovine enteric), E58 (bovine encephalomyelitis) was sequenced in an at-
tempt to define the relationship of this species to C. psittaci (ovine abortion and avian isolates), C. pneumoniae and C. trachomatis. Analysis of these data

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allowed the identification of restriction enzymes that clearly distinguish *C. psittaci* and *C. pecorum*.

2. Materials and methods

2.1. Chlamydial strains

Details of the chlamydial strains analysed in this study are shown in Table 1. All strains were propagated in developing chick embryos as previously described [11].

2.2. DNA extraction

DNA was extracted from yolk sac material as described by Anderson et al. [9]. Briefly, 100 μl of yolk sac was mixed with 400 μl of lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1% SDS and 200 μg/ml proteinase K) and incubated at either 37°C overnight or at 55°C for 2 h. The DNA samples were extracted twice with phenol:chloroform:isoamylalcohol, ethanol precipitated and resuspended in distilled water.

2.3. PCR protocol

PCR was used to amplify a 640 bp DNA fragment using 0.2 μM of primers 646C 5'-GTTGAGGGAGAGTCTATGGGATATCA-3' and 611C 3'-TACGACGGTAGGTGAGACTATCCAC-5' [9]. Generally, 2 μl of DNA was used in PCRs in a final reaction volume of 50 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X, 2 mM MgCl₂, 200 μM of each of the deoxyribonucleoside triphosphates and 0.5 units of Taq polymerase (Promega). The thermal cycler programme used for PCRs was 1 min 30 s at 94°C, 1 min at 45°C and 2 min at 72°C for 35 cycles.

Amplification of DNA that was subsequently cloned and sequenced was carried out using pfu DNA polymerase (Stratagene) in 100 μl reaction volumes containing 20 mM Tris-HCl (pH 8.5), 10 mM (NH₄)₂SO₄, 100 μg/ml bovine serum albumin, 0.1% Triton X-100, 200 μM of each of the deoxyribonucleoside triphosphates and 0.5 μM of 646C and 611C.

2.4. Dideoxynucleotide chain termination sequencing

PCR products were purified using Nucleiclean (Sigma) according to the manufacturer's instructions. Purified DNA was cloned into a PCR-Script® SK(+) vector (Stratagene) according to the manufacturer's instructions. Positive clones containing inserts were identified and plasmids were purified as described by Maniatis et al. [12]. Sequencing was carried out using the Sequenase Version 2.0 kit (USB) according to the manufacturer's instructions with some modifications. Briefly, plasmid DNA was alkali denatured using 0.1 volumes of 2M NaOH, 2M EDTA, incubated at 37°C for 30 min after which the DNA was neutralized by the addition of 0.1 volumes of 3M sodium acetate (pH 4.5), precipitated with 2 volumes of 100% ethanol and washed with 70% ethanol. This DNA preparation was used as template in primer annealing reactions containing 10% DMSO [13]. 1× sequenase buffer (40 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 50 mM NaCl) and O.2 μM of either 646C or 611C. Reactions were heated to 65°C for 2 min and allowed to cool slowly to room temperature. The labelling and termination reactions were carried out according to the manufacturer's instructions.

Partial 16S sequences of *C. psittaci* isolates S26/3, P94/1 and *C. pecorum* isolates W73, E8 were aligned and compared to *C. psittaci* 6BC [14] and EAE [15]. *C. pneumoniae* IOL-207 [6] and *C. trachomatis* strain 434 (unpublished observations by W.G. Weisburg, T.P. Hatch and C.R. Woese; GenBank accession no. M59178) using the computer programme MacMolly Tetra. RE analysis of sequences was also carried out using this computer programme.

2.5. Restriction enzyme analysis

Purified PCR products were digested using *BstI*...
and MaeI restriction enzymes (New England Biolabs) according to the manufacturer's instructions. DNA fragments were separated on 10% polyacrylamide gel and silver stained as described by Anderson et al. [9].

3. Results and discussion

The partial 16S nucleotide sequences of C. psittaci isolates S26/3, P94/1 and C. pecorum isolates E58 and W73 (GenBank accession numbers U61766 to U61770) were aligned and compared to previously published sequences of C. psittaci EAE [15], 6BC [14], C. pneumoniae IOL-207 [6] and C. trachomatis strain 434 (unpublished observations by W.G. Weisburg, T.P. Hatch and C.R. Woese; GenBank accession no. M59178) (Fig. 1). Analysis of these data indicate very high levels of sequence conservation, with the four species sharing greater than 94% sequence homology. In general, C. psittaci isolates shared approximately 98%, 96.6% and 95% identity to C. pecorum, C. pneumoniae and C. trachomatis isolates, respectively. Sequence data of C. psittaci 6BC 16S (1,500 nucleotides) showed 95.49% identity to C. trachomatis and 96.19% identity to C. pneumoniae [6,14] which are comparable to results obtained from the analysis of partial 16S RNA sequences determined in the present study. The high level of interspecies sequence conservation within the 16S RNA gene indicate a common ancestral lineage, with C. psittaci being more closely related to C. pecorum than to C. pneumoniae or C. trachomatis. These results are consistent with biochemical evidence which indicates that C. trachomatis is the only species of chlamydiae that is susceptible to sulfadiazine and capable of accumulating glycogen in inclusion bodies [3,16]. C. psittaci and C. pecorum grow readily in the yolk sac of chick embryos and possess round elementary bodies [3]. C. pneumoniae elementary bodies are typically pear-shaped [17].

Restriction enzyme analysis was carried out on the nucleotide sequences of C. psittaci and C. pecorum isolates. It was found that BsiI digestion of DNA amplified from C. psittaci and C. pecorum isolates yielded fragments of 540, 100 bp (Fig. 2; lanes 1, 2, 3) and 310, 230, 100 bp (lanes 4, 5, 6), respectively clearly distinguishing both species. Previous studies have relied on minor differences in the migration patterns of DNA fragments generated using MaeI to digest amplified DNA from the same region of both species [9]. From the sequence analysis carried out in the present study, both C. psittaci (abortion) and C. pecorum isolates possessed identical MaeI restriction enzyme sites. Differentiation of C. psittaci isolates S26/3 (abortion) and P94/1 (pigeon) was carried out using MaeI, generating DNA fragments of 60, 100, 130, 140 and 210 bp and 60, 130, 140, 310 bp (Fig. 2; lanes 7, 9), respectively. However, MaeI digestion of DNA amplified from C. psittaci VF88/

Table 1

Details of C. psittaci and C. pecorum isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Host</th>
<th>Clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S26/3*</td>
<td>Sheep</td>
<td>Abortion</td>
</tr>
<tr>
<td>P94/1</td>
<td>Pigeon</td>
<td>Ornithosis</td>
</tr>
<tr>
<td>VF88/2122</td>
<td>Budgerigar</td>
<td>Psittacosis</td>
</tr>
<tr>
<td>W73</td>
<td>Sheep</td>
<td>Normal (faeces)</td>
</tr>
<tr>
<td>C95/38</td>
<td>Sheep</td>
<td>Normal (faeces)</td>
</tr>
<tr>
<td>E58*b</td>
<td>Cattle</td>
<td>Encephalomyelitis</td>
</tr>
</tbody>
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b American Type Tissue Collection VR628.
2122 (budgerigar isolate) yielded an identical restriction profile to that obtained from C. psittaci S26/3 (lane 8). A similar result would also be expected to be obtained using DNA amplified from C. psittaci 6BC based on previously published sequence data [6]. These results suggest that even though the psittacine and non-psittacine avian isolates are very closely related, the psittacine strain is more closely related to the abortion isolate S26/3 than is the non-psittacine pigeon isolate. Andersen [18] reported that psittacine and non-psittacine turkey, duck and pigeon isolates can be distinguished using RE analysis of chromosomal DNA and reactivity to specific monoclonal antibodies.

The partial 16S RNA sequences determined in this study enabled the identification of restriction enzymes which clearly distinguish C. psittaci and C. pecorum isolates (BstI). Psittacine and non-psittacine (pigeon) isolates were distinguished using MacI. Using this system, isolates can be rapidly screened which will help to increase our understanding of the relationships between mammalian and avian chlamydial infections.

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References