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PHYSICAL MAPPING OF THE *COXIELLA BURNETII* GENOME

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Summary. - *Coxiella burnetii* isolates from different genomic groups contain restriction fragment polymorphisms that were easily distinguishable using pulsed field gradient electrophoresis (PFGE). Conversely, isolates that belong to the same genomic group yield identical patterns indicating that PFGE can be used to identify the genomic grouping of new *C. burnetii* isolates. Intact *C. burnetii* cells were embedded in agarose and lysed *in situ*. The genomic DNA was digested with low-frequency cutting restriction endonucleases, and subjected to PFGE analysis. *Not*I and *Sfi*I cut *C. burnetii* DNA least often and produced the largest fragments. *Apa*I, *Mlu*I, *Sal*I, *Xba*I or *Xho*I produced only small DNA fragments (±50 kbp). When PFGE was used to analyse *C. burnetii* genomes for the presence of plasmid-related sequences, all the plasmid sequences in Nine Mile and Priscilla were associated with their 36 kbp or 39 kbp plasmid bands, respectively. If these isolates contained plasmid sequences which had integrated into their chromosomes those sequences would have been visible as additional bands. These same studies also showed that plasmid sequences in the plasmidless-Ko isolate were completely contained within two *Not*I fragments, indicating that the integrated plasmid is localized to a concise region of the *C. burnetii* genome. Since it is difficult to conduct genetic analyses of obligate intracellular parasites using standard techniques, a physical map is being developed using PFGE. In addition to providing a means for determining gene loci, the physical maps provide a means for comparing genetic organization among the different strains of *C. burnetii*.

Key words: Coxiella burnetii; rickettsiae; pulsed field gradient electrophoresis; restriction mapping

Introduction

It is now possible, using PFGE methods, to separate DNA fragments as large as nine megabase pairs (Mbp) (Schwartz and Cantor, 1984; Smith and Cantor,

1987). In this procedure, whole *Coxiella burnetii* cells were embedded in agarose, the cells were lysed *in situ,* and the released DNA was digested with low-frequency cutting restriction endonucleases. The subsequent electrophoretic fractionation of DNA restriction fragments provides a rapid method of assigning *C. burnetii* organisms to genomic groupings. This technique can also be used to monitor their entire genomes for large chromosomal rearrangements (Smith *et al.,* 1986). This procedure can also be used to develop physical maps of the various *C. burnetii* genomes (Smith *et al.,* 1987). Since it is difficult to conduct classic genetic analyses of obligate intracellular parasites the presence of physical maps provides an alternative means of deriving genetic maps for these organisms.

Materials and Methods

Coxiella burnetii isolates were propagated in embryonated hens' eggs and purified *C. burnetii* were suspended in 0.01 mol/l Tris, 0.001 mol/l EDTA (TE buffer) at pH 7.5 at a concentration of $2x10^9$ cells/ml and diluted with an equal volume of low-melting agarose at 37 °C. The suspension was placed into 100 μl forms and allowed to cool on ice for 5 min. Solidified blocks of agarose were transferred to a lysis mixture containing about 2 volumes of ES buffer (0.5 mol/l EDTA at pH 7.5 and 1 % SDS). After incubation at room temperature for 1 hr, proteinase K was added to 0.5 mg/ml, and the mixture was incubated overnight at 55 °C. The blocks were washed twice in 10 volumes of TE for 30 min at room temperature and then twice for 1 hr each at 50 °C in TE buffer + 0.04 mg/ml phenylmethylsulfonylfluoride to inactivate the proteinase K. After two more TE washes at 37 °C (using 5 volumes of buffer to 1 volume of gel plugs), the blocks were stored in ES buffer at 4 °C. One block, containing 3 to 10 μg of DNA, was used for each restriction digest with restriction endonucleases that are known to cut DNA infrequently (*Apa*I, *Mlu*I, *Not*I, *Sal*I, *Sfi*I, *Xba*I, and *Xho*I). Before digestion with these restriction enzymes, agarose blocks were rinsed twice for 30 min with TE. Digestion was carried out according to the manufacturer's instructions. The plugs were incubated for 3 hr at the appropriate temperature in a sterile 1.5-ml microfuge tube containing 500 μl of reaction buffer containing enzyme (50 units/tube) and two gel plugs. The enzyme-buffer solution was then decanted, and the digestion step was repeated for a total of three incubations. When multiple enzyme digestions were employed, they were conducted in two stages, using the enzyme that requires the lowest salt or temperature first (*Not*I is incubated at 37 °C in low salt and *Sfi*I was incubated at 50 °C in high salt). Digestion was stopped by aspirating the restriction buffer and adding 1 ml of ES buffer. After incubating at 50 °C for 2 hr, the ES buffer was removed, and 1 ml of ESP buffer (0.5 mol/l EDTA, 0.5 mg/ml proteinase K at pH 7.5, and 1 % SDS) was added. The plugs can be stored at 4 °C for several months (Heinzen *et al.,* 1990). The data shown here were generated using either the CHEFDR™ (Bio Rad) pulsed field electrophoresis apparatus or the Geneline™ (Beckman) device. Running gels were prepared using 1.0 % to 1.5 % Ultrapure Agarose (BRL) in 0.5X TBE buffer at pH 8.0. Electrophoresis was in 0.5X TBE buffer at about 14 °C. The buffer was changed every 24 hr during long runs.

Results and Discussion

The largest fragments of *C. burnetii* DNA remaining after digestion with *Apa*I, *Mlu*I, *Sal*I, *Xba*I or *Xho*I were less than 50 kbp. The restriction enzymes *Not*I and *Sfi*I cut *C. burnetii* DNA less often and produced larger fragments

(Table 1). As a result those enzymes have been used to examine several *Coxiella* isolates for restriction fragment length polymorphisms. Size polymorphisms were apparent among the various. *C. burnetii* isolates analysed in these gels. Based on genetic and phenotypic differences in *Coxiella* isolates, it is no longer appropriate to classify these organisms together. There are at least six different strains or genomic groups of *Coxiella* (Samuel *et al.*, 1988; Heinzen *et al.*, 1990; Mallavia *et al.*, 1991). The Nine Mile, Priscilla, S, and Dugway isolates, all from different strains or genomic groups (Hamilton, Biotzere, Corazon, and Dod strains, respectively), were easily distinguishable in these gels. Conversely, the S, Ko, G, and L isolates, all Corazon strains, gave identical patterns (Fig. 1). Since the genomic groupings derived with this method are consistent with genomic groupings derived using other procedures, such as restriction fragment length polymorphism and lipopolysaccharide analyses (Samuel *et al.*, 1983; 1985; Hackstadt, 1986; Moos and Hackstadt, 1987), this method can be used to identify the genomic grouping of new *C. burnetii* isolates (Heinzen *et al.*, 1990). However, it is important to realize that the different strains represent diversity in both plasmid sequences and in chromosomal sequences. Specifically, different plasmids are found in combination with distinct genomic DNA sequences.

The Nine Mile isolate contains QpH1 plasmid, Priscilla has the QpRS plasmid, and the Ko isolate has plasmid sequences integrated into its genome. Supercoiled plasmids do not migrate in these gels and *Sfi*I does not cut these plasmids, but *Not*I linearizes all of them. When the DNA from Nine Mile and Priscilla was digested with *Not*I their plasmids migrated as linear DNA. Upon

Table 1. DNA fragments resulting from restriction digestion and PFGE analysis of *C. burnetii* strains

	DNA restriction fragments			
	*Not*I fragments		*Sfi*I fragments	
Strain (isolate)	Number	Largest (kb)	Number	Largest (kb)
Hamilton (Nine Mile)	19	250	19	370
Biotzere (Priscilla)	20	290	15	320
Corazon (S)	16	260	17	410
Dod (Dugway)	16	240	16	380

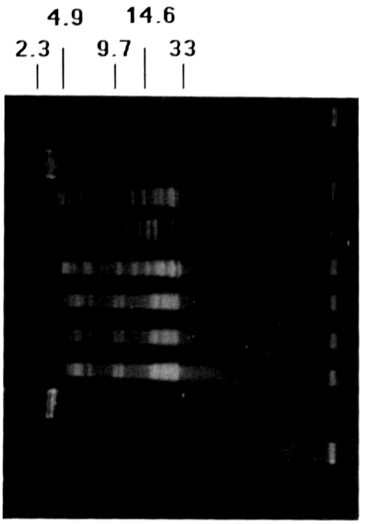


Fig. 1

PFGE separation of *Not*I digest of six different isolates of *Coxiella burnetii*
Electrophoresis conditions were 150 V, 14 °C, in 1 % agarose in 0.5X TBE, 22 hr at 6 sec and 22 hr at 15 sec. Molecular size standards are based on lambda DNA concatemers and *Hind*III-digested lambda DNA. The gel was stained with ethidium bromide and photographed using UV to illuminate bands. These conditions provided good resolution and accurate size estimations of larger DNA fragments.

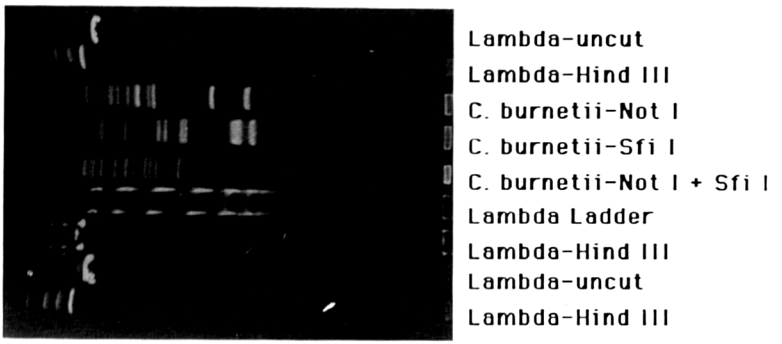
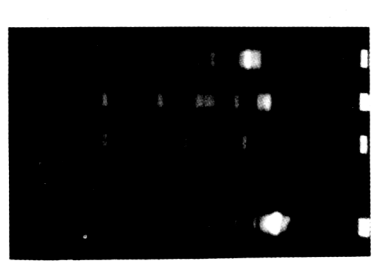


Fig. 2

PFGE separation of *Not*I, *Sfi*I, and *Not*I plus *Sfi*I digestion of the Priscilla isolate of *Coxiella burnetii*
Concatemers of lambda, undigested lambda and *Hind*III digested lambda DNA serve as molecular weight markers. Switch time, 30 sec for 40 hr. Electrophoresis was at 150 V, 14 °C, in 1 % agarose in 0.5X TBE; the gel was stained with ethidium bromide and photographed using UV to illuminate band fragments.

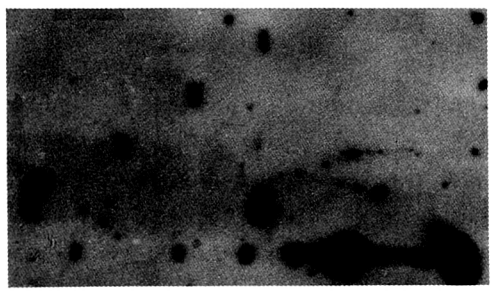


Lane
1 Nine Mile *Not I*
2 Nine Mile *Sfi I*
3 Nine Mile *Not I + Sfi I*
4 Lambda *Hind III*
5 Lambda ladder

1 2 3 4 5

Size Markers
Lambda Units
(X 48.5 bp)

A



Lane
1 Nine Mile *Not I*
2 Nine Mile *Sfi I*
3 Nine Mile *Not I + Sfi I*
4 Lambda *Hind III*
5 Lambda ladder

Size Markers
Lambda Units
(X 48.5 bp)

B

Fig. 3

A. PFGE separation of *Not*I, *Sfi*I, and *Not*I plus *Sfi*I digestion of the Nine Mile isolate of *Coxiella burnetii*. Concatemers of lambda DNA serve as molecular weight markers.

B. Southern analysis of PFGE gels was used to map the *glt* A gene to a 180 kbp *Not*I fragment, a 110 kbp *Sfi*I fragment and a 70 kbp *Not*I - *Sfi*I fragment from the Nine Mile strain of *Coxiella burnetii* genome. This also provided a *Not*I - *Sfi*I map of 220 kbp of the genome. The gel was hybridized with a ^{32}P-labelled cloned fragment of the *glt* A gene.

Table 2. Characterization of DNA fragments from *Not*I restriction digestion and PFGE analysis of the Priscilla isolate of *Coxiella burnetii*

Fragment number	Size of fragment (kb)
1	293
2	223
3	150
4	135
5	121
6	96
7	87
8	78
9	74
10	62
11	52
12	43
13	39 (nicked plasmid)
14	33
15 and 16	28 (doublet)
17	19
18	16
19	15
20	10

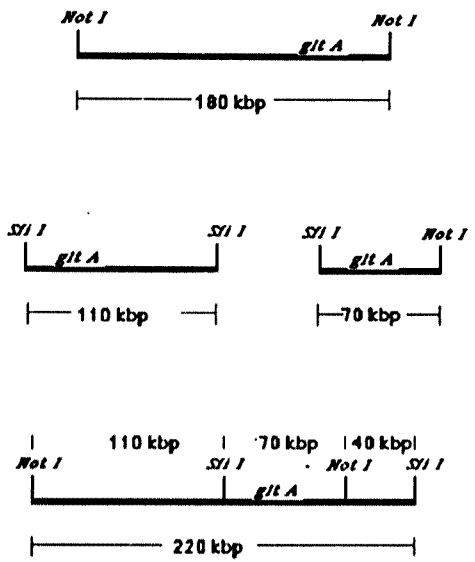


Fig. 4
Mapping of the *glt* A locus within the *Coxiella* (Nine Mile strain) genome

Southern blot analysis (Southern, 1975) all *Coxiella* plasmid sequences in the Nine Mile isolate migrated as a ~36 kbp fragment and at ~39 kbp in Priscilla (Samuel *et al.,* 1985). In the Ko strain the integrated plasmid sequences are detected in two DNA fragments one at ~39 kbp and the other at ~100 kbp. Further, neither the Nine Mile or the Priscilla isolates contained plasmid sequences integrated in their genomes, since additional sequences were not visible as distinct bands in the autoradiographs (Heinzen *et al.,* 1990).

The DNA fragments generated by these methods can be used to size the genome of various isolates (Fig. 2) and to develop physical maps of the *C. burnetii* genomes. Summing the M_r of either *Sfi*I or *Not*I fragments (Table 2) indicates that the *C. burnetii* genome of both Hamilton and Biotzere strains is about 1.6×10^6 bp, a value consistent with renaturation kinetics data (Myers *et al.,* 1980). The DNA fragments generated by these methods can also be used for mapping *C. burnetii* genes as illustrated in this paper using the *glt* A locus (Fig. 3). In this procedure the electrophoresed DNA fragments are probed to determine the location of a gene to specific DNA restriction fragments. Using this information a partial map of the *glt* A locus has been derived (Fig. 4). This technique can also be used for comparing gene loci and genetic organization among the different strains of *C. burnetii.*

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