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Characterization of IS*1501* mutants of *Leptospira interrogans* serovar pomona

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Abstract

Leptospira interrogans is a diverse species in which individual serovars have distinctive restriction fragment length polymorphisms that are useful in strain identification. Many of these polymorphisms can be detected using hybridization probes derived from insertion sequences; an observation that suggests these IS elements are active and can transpose in *L. interrogans*. Two spontaneous mutants of *L. interrogans* serovar Pomona strain RZ11 were isolated by immune selection and characterized. Changes in the size and antigenicity of LPS from these mutants were detected. Genetic analysis showed that both mutants have additional copies of an IS*3*-like element, designated IS*1501*, that are not present in the parental strain. One mutant, GT211, has a single additional copy of IS*1501*, whereas the other mutant, GT210 has three additional copies of IS*1501* relative to strain RZ11. IS*1501* transposition generated 3-bp direct repeats from target sequences flanking the insertion site. RT-PCR analysis of transcripts at altered loci showed IS*1501* transcripts extended into adjacent sequences. These data are the first to show spontaneous transposition of an endogenous *Leptospira* insertion sequence, and suggest that IS*1501* may be capable of gene activation.

Keywords: Spirochete; Insertion sequence; Transposition

1. Introduction

Leptospira interrogans is one of several species that causes leptospirosis, one of the most widespread zoonotic diseases in the world [1]. Leptospirosis persists by chronic infection of mammalian maintenance hosts that exhibit few clinical signs of disease. Passage to non-maintenance hosts can result in a wide range of clinical symptoms, ranging from a mild disease to an acute infection resulting in multiple organ failure and death [2]. *L. interrogans* is a diverse species with many antigenic types (serovars), each having its preferred maintenance host [2].

Genetic diversity among *L. interrogans* isolates can often be detected by identifying serovar-specific restriction fragment length polymorphisms (RFLPs) using probes to endogenous insertion sequences (IS) [3]. Insertion sequences are mobile genetic elements that can disrupt genes by insertion or alter gene organization via chromosomal rearrangements [4]. IS-specific RFLPs may arise by transposition or recombination events. There are several different types of IS elements in *L. interrogans* and large rearrangements within the genome coincide with the locations of some of these elements [5–7]. Although the copy number and distribution of

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endogenous IS elements varies between serovars, transposition of these elements to new sites has not been demonstrated in *L. interrogans*.

Genetic analysis of pathogenic *Leptospira* has been impeded by the lack of tools to create mutants, complement genes, or develop selectable markers. Several investigators have used immune selection as a means to isolate *Leptospira* strains with altered phenotypes [8–13]. In this study, immune selection was used to isolate *L. interrogans* mutants, and the mutants were characterized. Additional copies of an IS*3*-like element, IS*1501*, were detected in two mutants, demonstrating that this element transposes in *L. interrogans*. Comparison of parental and mutant loci showed that transcripts from within IS*1501* extended into sequences adjacent to the insertion site.

2. Methods

2.1. Bacterial strains

L. interrogans serovar Pomona strain RZ11 was colony purified on solid EMJH media [14–16] and propagated at 29 °C. *Escherichia coli* INV α' (Invitrogen Corp.) was propagated in DYT medium [17]. When needed, kanamycin was used at a final concentration of 50 μg ml^{-1}.

2.2. Immune selection of mutants

Spontaneous mutants of strain RZ11 were selected by growth in the presence of immune sera prepared in rabbits against *L. interrogans* serovar Pomona and fresh guinea pig serum (as a source of complement), each at a final dilution of 1:100. Bacteria were transferred to fresh media containing 1:50 dilutions of immune rabbit and fresh guinea pig sera, followed by two passages in media containing immune rabbit sera alone. Each passage was carried out for 1 week. Bacteria were passed twice in media lacking immune sera, and the antigenicity of cultures was determined using the microscopic agglutination test (MAT) following established techniques [18]. Strains were colony purified on solid EMJH as described previously [14].

2.3. Analysis of Leptospira outer membrane components

Outer membrane (OM) fractions were prepared from *L. interrogans* cells as described [19,20]. SDS–polyacrylamide gel electrophoresis, staining, Western immunoblotting, and immune detection were performed as previously described [19]. LPS was stained with silver as described by Tsai and Frasch [21]. LPS was prepared from detergent extracts using a modification of the method described by Hitchcock and Brown [22], whereby OM samples were treated with proteinase K (0.5 μg per 1 μg OM protein) for 1 h at 37 °C. Immune sera were prepared in New Zealand white rabbits using OM fractions mixed with adjuvant according to the manufacturers instructions (Corixia Corp.).

2.4. DNA techniques

Methods for genomic DNA isolation, agarose gel electrophoresis, Southern blotting, and hybridization analysis were performed as previously described [14]. Hybridization probes corresponded to the complete IS*1500* element [5] and the first 656 bp of IS*1501* [Accession number AF038932].

Sequences adjacent to IS insertions were amplified by a genome walking technique as described previously [23] using IS*1501*-specific primers 1501-G3p and 1501-G5p paired with a linker specific primer AP1 (Table 1). PCR products were either cloned into pCR2.1 (Invitrogen Corp.) or gel purified prior to sequencing.

PCR primers that anneal to sequences flanking mutant-specific IS*1501* insertions (Table 1) were used to amplify homologous sites from samples of RZ11, GT210, and GT211 DNA. PCR reactions were done using AccuTaq (Sigma–Aldrich Corp.) with the program: 94 °C, 2 min, followed by 37 cycles of 94 °C for 15 s, 60 °C for 30 s, and 68 °C for 7 min.

DNA sequencing was performed by dye termination reactions separated on ABI Prism sequence analyzers at the Iowa State University Nucleic Acid Facility (Ames, IA) and on an ABI 3700 at the NADC Genomics Laboratory. Sequence data were complied using Sequencher v. 4.1 (GeneCodes), and the data analyzed using Clone Manager 7 and Primer Designer 5 (Scientific and Educational Software), BLAST [24], and BPROM (http://www.softberry.com/). New sequence data was submitted to GenBank under the accession number AY919671.

2.5. RNA techniques

Total genomic RNA was isolated from *L. interrogans* and analyzed by reverse-transcriptase (RT) PCR reactions as previously described [25] using primers listed in Table 1. Each RT PCR reaction was paired with an identical reaction lacking reverse transcriptase to confirm that residual genomic DNA had been removed.

3. Results and discussion

3.1. Immune selection and initial characterization of L. interrogans mutants

Immune selection is useful in obtaining mutants of *Leptospira* having altered antigenic compositions