Comparison of Flanking Regions of the 5S Ribosomal Ribonucleic Acid Genes in *Leptospira biflexa* and *Leptospira interrogans*

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One of the genes encoding the 5S ribosomal ribonucleic acid (rRNA) for the *Leptospira biflexa* strain Patoc I was isolated and sequenced. The physical maps of the 5S rRNA genes in *Leptospira* were constructed. The strains of *Leptospira biflexa* had two genes on their chromosome; these two 5S rRNA genes were located several kb apart and sequences flanking these genes were divergent. In contrast to saprophytic leptospires, maps in parasitic leptospires that had only one gene for 5S rRNA on their genome were highly conserved and the physical maps of the genes in almost all strains were similar.

Keywords *Leptospira biflexa*; *Leptospira interrogens*; 5S rRNA gene; DNA sequence; cloning; physical map

Introduction

Leptospires are easily distinguishable from other eubacteria because of their unique morphological characteristics. The genus *Leptospira* is a physiologically and genetically distinct group of eubacteria[1,2] and is composed of two species, *L. interrogans* and *L. biflexa*. Strains of *L. interrogans* are parasitic for humans and animals, and those of *L. biflexa* are saprophytic.[3,4]

We have been studying the molecular biology of *Leptospira* with special emphasis on ribosomal ribonucleic acid (rRNA) gene organization, since this is a peculiar gene organization. As we have reported, three kinds of rRNA genes in *Leptospira* were unlinked and were transcribed independently.[5,6] This is a remarkable feature in the organization of the genes. Furthermore, the number of 5S rRNA genes depended on parasitism, whereas there are two larger rRNA genes in both species.[7,8]

Very little is known about the genetics of these bacteria because of the difficulty in studying them using classical genetic means.[3] The results of our study suggest that *L. biflexa* genes are probably organized differently from corresponding genes in *L. interrogans*. In this report we present the nucleotide sequence of *L. biflexa* 5S rRNA genes and the physical maps of flanking regions of these genes in their genome.

Materials and Methods

Bacterial Strains The bacterial strains were kindly provided by R. C. Johnson, H. Korver and W. J. Terpstra. The leptispires were cultivated and harvested as described previously.[8]

Deoxyribonucleic Acid (DNA) Preparation and Southern Blot Hybridization Total cellular DNA was extracted by the method described in previous papers.[5,7] DNAs were cloned by the standard method of Sambrook *et al.*[9] and DNA probes were also prepared as described previously.[7] DNA fragments in agarose gels were transferred and hybridized to the probes. Experimental conditions were as described in our previous paper.[6]

Dideoxy Sequencing Ordered deletion mutants were generated by digestion with exonuclease III and sequenced by the dideoxy-chain termination method.[10]

Results and Discussion

Cloning and Sequencing of the 5S rRNA Gene for *L.*

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TTTGATATCA GCATCACCAG GTGCGTTGGA AGTCCGGATT TTAAGACCCA TCACCGTGAA   60
   EcoRV
TTTTTCAGTT TTTACCAATG GTTCCATCAG TTCCGACATA GAGAAAAAGT TTCAGCTTCC  120

TAAAAAAACG CAACCTTTTC TTACTGAAAG GTTTGACTTA CTGAACCTCT CCGTACATAT  180
                                    -35                      -10
GGTAATTACT CCTGGTGATT ATGGAGAGAA GGCCATACCC GTTCCCATTC CGAACACGGA  240

AGTCAAGCTT CTCATCGCCG ATGGTACTAT TGGGTTCGCT CAATGGGAGA GTAGGACATT  300
   HindIII              5S rRNA gene
GCCGGGTTAG CACTAATAAC TCTTTAGAAA GCGTTCACGA AAGTGAGCCG TTTTTTTGTT  360

TGTGGAGCGT TTTGTGATCC GGCTAATGTC CGCGACTTTT GGCTACGTCC GCTGCTTCGC  420

CCGCCAAAGT CGCCTGCTCG TTCCCTATGG GTCACTGCGC AGGATTGCGG TTAAAATCTT  480

TTTCTTATCT ATTAATATAA AAAACCCAAA TCATACCGCT AGTCACCAGT TGTTCGCGAA  540
                                                            NruI
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Fig. 1. Nucleotide Sequence of the 5S rRNA Gene and Its Flanking Regions for *L. biflexa* Strain PatocI

The 4.3-kb *Eco*RI fragment was cloned into bacteriophage lambda gt11 and then subcloned into the pUC18 *Eco*RI site. Deletions were made by exonuclease III digestion followed by mung bean nuclease digestion. All sequences were determined both strands for dideoxynucleotide terminating sequencing as described by Sanger *et al.*[10] The mature 5S rRNA sequences are boxed and putative promoter sequences are underlined. The nucleotide sequence reported in this paper will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number D90255.

Table I. Sizes of Restriction Endonuclease Digested Genomic DNA Fragments Hybridized with the 5S Gene Probe[a)]

DNA fragment (kb) digested by:	*Leptospira biflexa* strain PatocI	CH11	Waz	Gent	A284	LT430
	Size of fragment in kilobases					
*Ssp*I	3.0	7.0	5.8	5.6	4.4	2.2
	1.5	3.3	3.1	1.1	1.4	1.4
*Hin*dIII + *Ssp*I	1.6	6.1	2.8	2.6	3.1	1.3
	1.5	1.6	2.4	0.6	1.2	0.8
	0.9	1.1	1.9	0.5	0.7	0.7
	0.6	0.6	1.0	0.4	0.6	0.7
*Eco*RI	4.3	8.2	4.2	3.0	3.8	5.4
	1.4	1.5	2.3	2.2	1.2	2.9
*Hin*dIII + *Eco*RI	3.4	1.6	2.5	1.7	2.0	3.0
	0.8	1.5	1.3	1.6	1.8	2.0
	0.7	0.8	0.9	0.6	0.6	1.5
	0.6	0.7	0.5	0.5	0.5	0.7
*Cla*I	6.9	4.0	2.8	2.4	3.0	10.0
	5.2	2.5	2.3	1.8	1.3	4.0
*Hin*dIII + *Cla*I	3.2	2.3	2.0	1.4	1.8	3.2
	2.7	1.6	1.5	1.0	1.0	2.9
	1.4	1.5	0.8	0.8	0.8	1.8
	1.3	1.0	0.7	0.7	0.6	1.5

a) Genomic DNA was digested with restriction endonuclease as indicated, electrophoresed in an agarose gel and blotted on a nylon membrane. The DNA fragment containing the entire 5S rRNA gene (*ca.* 500 bases) was labeled with [^{32}P]deoxycytidine 5′-triphosphate using a random primer labeling kit (Takara Shuzo Co., Ltd., Kyoto) and used as a hybridization probe. The molecular sizes of hybridization fragments in kilobases were calculated using *Eco*T14I digests of lambda phage DNA as a molecular size marker.

***biflexa* Strain Patoc I** One of the 5S rRNA genes in the *L. biflexa* strain Patoc I is known to be located on the 4.3-kb *Eco*RI fragment.[6)] Therefore, a lambda gt11 gene bank of strain Patoc I was constructed and recombinant bacteriophages containing the 5S rRNA gene were isolated by plaque hybridization as described previously.[7)] The inserted leptospiral DNA fragment was excised, and subcloned into pUC18 vector. The restriction map was constructed and ordered deletion mutants were made using standard procedures.[5,9)] The nucleotide sequences were then determined by the method of Sanger *et al.*,[10)] and resulting sequences are shown in Fig. 1. The 5′ and 3′ termini were assigned according to the homologous sequence of *L. interrogans* strain Moulton[5)] and bases encoding the 5S rRNA are boxed in Fig. 1. The coding region of the gene was found to be 117 nt long and there were 14 substitutions in comparison with the gene for strain Moulton. We have reported that the invaluable 5S gene of *L. interrogans* has own promoter for its transcription. Putative promoter sequences resembling the strain Moulton are underlined in the figure.

Southern Hybridization Analysis of 5S rRNA Genes in *L. biflexa* Some of the strains of Leptospira (Patoc I and Urawa) have been studied and the hybridization patterns of the gene probe have been reported.[6)] Five more strains were examined in this study. Hybridization with the 5S probe of all the strains used gave two radioactive bands and some of the results are shown in Table I. Hybridization experiments in double digests showed four radioactive bands of approximately equal intensity. These results indicated that there is a *Hin*dIII cleavage site in the 5S rRNA gene and that the sequence of the gene is highly conserved in these strains.

Physical maps of the 5S rRNA genes in the strains

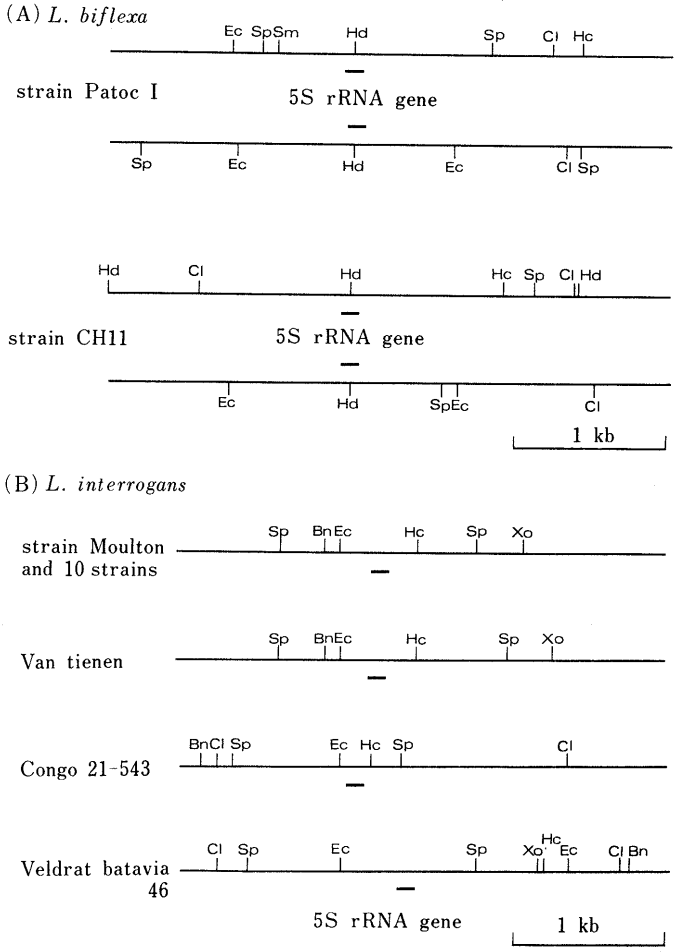

Fig. 2. Physical Maps of the 5S rRNA Gene and Its Flanking Regions for the Strains of *L. biflexa* and *L. interrogans*

A) Genomic DNA was cleaved with restriction enzymes (double or triple digestion) and electrophoresed in a 2% agarose gel. Three types of probes were used for hybridization experiments. The DNA fragment containing the entire 5S rRNA gene, the *Eco*RV—*Hin*dIII fragment or the *Hin*dIII—*Nru*I fragment was labeled and used as a probe. The sizes of the fragments were calculated and physical maps were constructed. B) Genomic DNA was digested, electrophoresed and blotted to a nylon membrane. The DNA fragment generated by *Eco*RI and *Hin*cII double digestion (*ca.* 450 bases) of recombinant plasmid DNA was labeled and used as a hybridization probe.[5)] Physical maps for the parasitic strains were established. 10 strains: Akiyami C (serogroup Australis), Akiyami A (Autaumnalis), Hebdomadis (Hebdomadis), RGA, Ictero No. I, Lai, Mankarso (Icterohaemorrhagiae), Pomona (Pomona), Salinem (Pyrogenes) and Hardjo (Hardjo). Restriction enzymes; Ec, *Eco*RI; Sp, *Ssp*I; Sm, *Sma*I; Hd, *Hin*dIII; CI, *Cla*I; Hc, *Hin*cII; Bn, *Ban*II; Xo, *Xho*I.

Patoc I and CH11 were constructed by Southern blot hybridization. Two different probes were used to establish these physical maps. The *Eco*RV—*Hin*dIII fragment (including the 5′ half of the gene) or *Hin*dIII—*Nru*I fragment (including the 3′ half of the gene) of the recombinant plasmid DNA was electroeluted from an agarose gel, purified, labeled and used as a probe. Genomic DNA digests with two or three enzymes were hybridized to the 5′ half or 3′ half probe. The results obtained in these experiments, in conjunction with those obtained in the genomic Southerns, allowed us to locate the sequences corresponding to the 5S rRNA with respect to the restriction maps shown in Fig. 2A. The gene number in both strains is thus two and the genes are located at least several kb apart. The sequences flanking regions of the gene were divergent.

Physical maps of the 5S rRNA gene on the leptospiral genome *L. interrogans* strain Moulton possesses only one

gene for 5S rRNA. A detailed hybridization patterns of the probe and restriction map for the strain Moulton has been already presented.[5] Limitation of the 5S rRNA gene to a single copy in parasitic leptospires is unknown, but this is a common feature in parasitic leptospires. Different hybridization patterns are observed in some strains. The heterologous existence of the gene in parasitic strains is correlated with the different biological characteristics of the strains.[4] Physical maps of the strains have been determined and are shown in Fig. 2B. Eleven out of 14 strains showed an identical map for the 5S rRNA gene, strain Van Tienen was quite similar to that of strain Moulton while two other strains (Congo 21-543 and Veldrat batavia 46) showed less similarity.

Though there are two copies of the gene encoding the 5S rRNA from saprophytic leptospires, why the 5S rRNA gene occurs as a single copy in parasitic leptospires is not known. In the present study, we showed that the maps flanking the 5S rRNA gene in most parasitic *Leptospira* are identical and the sequences are well conserved. We have also shown that saprophytic leptospires have two 5S rRNA genes and that the sequences flanking the genes are conserved to a lesser degree.

The spirochetes, including the genera *Leptospira* and *Leptonema*, are one of the relatively ancient evolutionary branches of the eubacteria.[1,11] Leptospires are now divided into several groups by their biological characteristics,[3,4] but they are descendants of the same progenitor prior to the divergence of the parasitic and saprophytic leptospires. Parasitic and nonparasitic leptospires are clearly distinguishable from each other by the number of 5S rRNA genes and comparison of restriction patterns of the regions flanking the genes. Thus, analysis of the organization of 5S rRNA genes can be beneficial in the taxonomic and phylogenetic studies of leptospires.

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