Penicillin-Binding Proteins in *Leptospira interrogans*

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The *Leptospira interrogans ponA* and *phpB* genes were isolated and characterized. *ponA* and *phpB* encode the penicillin-binding proteins (PBPs) 1 and 3, respectively. There is little sequence variation between the PBP genes from two *L. interrogans* strains (serovar icterohaemorrhagiae strain Verdun and serovar pomona strain RZ111). The deduced *L. interrogans* PBP 1 and PBP 3 protein sequences from the two strains shared over 50% similarity to homologous proteins from *Escherichia coli*. It was demonstrated for strain Verdun that *ponA* and *phpB* are transcribed individually from their own promoter. The *ponA* and *phpB* genes from both strains are separated by 8 to 10 kb and oriented such that their transcription is convergent. The *L. interrogans* PBP 1 and PBP 3 proteins were synthesized in *E. coli* and were modified with ampicillin using a digoxigenin-ampicillin conjugate. These data show that both PBP genes encode functional PBPs.

Leptospirosis is a widespread zoonosis caused by *Leptospira interrogans*. This bacterial pathogen can infect most mammalian species through either direct or indirect contact with contaminated body fluids from an infected animal (7). Leptospirosis can be fatal in humans. In livestock, *Leptospira* infection may result in death or a chronic infection may ensue, leading to abortion, stillbirth, infertility, or decreased milk production. *Leptospira*-infected humans are often treated with β-lactam antibiotics. It has recently been suggested that leptospirosis in livestock can be treated with β-lactam antibiotics (19). In vitro, pathogenic leptospires are very sensitive to β-lactam antibiotics (16). The MIC of ampicillin is between 0.025 and 0.78 μg/ml, and that of penicillin G is between 0.39 and 3.13 μg/ml. The minimal bactericidal concentrations observed for penicillin G are up to 100 μg/ml or more. In contrast, ampicillin exhibits high bactericidal activity, as evidenced by low minimal bactericidal concentrations (<25 μg/ml).

β-Lactams exert their effects by acting as substrate analogs of the peptidoglycan biosynthetic enzymes transpeptidase and β-alanine carboxypeptidase (21). These enzymes are located within the cytoplasmic membrane and play an integral role in the synthesis of peptidoglycan. These proteins are commonly called penicillin-binding proteins (PBPs) because of their ability to covalently bind radiolabeled penicillin (20). There are two distinguishable groups of PBPs: low-molecular-weight PBPs and high-molecular-weight (HMW) PBPs. The low-molecular-weight PBPs are monofunctional enzymes acting as β-carboxypeptidases involved in the remodeling of peptidoglycan during cell growth. The HMW PBPs have a multidomain structure. These proteins are anchored to the cytoplasmic membrane by an N-terminal pseudo-signal peptide and are essentially composed of two modules localized on the outer face of the cytoplasmic membrane. The N-terminal domain, which is several hundred amino acids long, is fused to the C-terminal penicillin-binding domain. This domain displays the transpeptidase activity that catalyzes cross-linking of the peptidoglycan peptides. Pairwise comparison and multiple alignments of amino acid sequences lead to the conclusion that HMW PBPs fall into two classes, A and B, which differ in their N-terminal domain (8, 13). In *Escherichia coli*, PBPs 1a and 1b of class A behave as bifunctional proteins exhibiting both transglycosylase (N-terminal module) and transpeptidase (C-terminal module) activities. They catalyze polymerization of the peptidoglycan from undecaprenyl diphosphate-linked di-saccharide peptides, probably by producing primers for PBP 2 and PBP 3 to act upon during cell elongation and cell division. PBP 2 and PBP 3 of class B are likewise considered bifunctional proteins, though the role of the N-terminal module is not clearly established. PBP 3 is specifically involved in polymerization of the septal peptidoglycan during cell division (14). Little is known about the PBPs of *Leptospira*. During analysis of subcellular fractions, five PBPs were identified in *Leptospira kirschneri* (10). However, neither the proteins nor the genes that encode them have been characterized.

To establish a framework by which leptospiral peptidoglycan structure can be analyzed, we isolated and characterized the *L. interrogans ponA* and *phpB* genes, encoding PBP 1 and PBP 3, respectively, which play an important role in peptidoglycan synthesis. Comparison of these sequences from two strains (serovar icterohaemorrhagiae strain Verdun and serovar pomona strain RZ11) also provides information on genetic drift between distinct serovars of the same species.

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**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** Bacterial strains and plasmids used are detailed in Table 1. *E. coli* strains were grown at 37°C in Luria-Bertani broth (18). Antibiotics and substrates were used in selective media at the indicated concentrations: isopropyl-β-d-thiogalactopyranoside (IPTG) at 500 μM, 5-bromo-4-chloro-3-indolyl-β-d-galactoside at 80 μg/ml, ampicillin (AMP) at 50 μg/ml, and kanamycin at 30 or 50 μg/ml. *L. interrogans* serovar icterohaemorrhagiae strain Verdun (National Reference Center for Leptospira, Institut Mole´culaire et Me´dicale, Institut Pasteur, 25 rue du docteur Roux, 75724 Paris Cedex 15, France. Phone: 33 (1) 45 68 83 66. Fax: 33 (1) 40 61 30 01. E-mail: isgirons@pasteur.fr.

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Pastore, Paris, France) and serovar pomona strain RZ11 (24) were grown in EMH medium at 28°C (6, 12).

Cloning and sequencing of the \( \text{ponA} \) and \( \text{pBPB} \) genes. The \( \text{ponA} \) gene of \( L. \) interrogans serovar pomona strain RZ11 was cloned from a previously described plasmid-based BamHI library (24) and identified during sequence analysis of randomly picked clones (4). The 3' end of this gene was amplified using a PCR-based genome walking technique (Universal Genome Walker Kit, Clontech, Palo Alto, Calif.) using the conditions described previously (25). Specific amplification of \( \text{ponA} \) was initiated with primer 173 (Table 2) on the 5' end of this gene (3.1-kb PCR product using primers 155 and 513) cloned in pCR2.1 vector. This paper.

For amplification of the \( L. \) interrogans strain Verdun locus, the cycling parameters were as recommended by the supplier to amplify 10- to 20-kb templates. Two additional primers, oligo5.4RP, which anneal to opposite strands of a 5.4-kb linker-specific primer AP1) cloned in pCR2.1 vector.

The \( \text{pBPB} \) and \( \text{pBPB3} \) genes from strains Verdun and RZ11. Long-distance PCR (LD-PCR) products were amplified from strain RZ11 genomic DNA using primers 921 and 922 cloned in pCR2.1 vector. This paper.

LD-PCR. PCR was used to determine the distance and orientation between the \( \text{ponA} \) and \( \text{pBPB} \) genes from strains Verdun and RZ11. Long-distance PCR (LD-PCR) products were amplified from strain RZ11 genomic DNA using \( \text{Tth} \) polymerase (Clontech) using the amplification parameters described previously (25) and primer 184, located downstream of \( \text{ponA} \), paired with either primer 185 or 186, oriented in opposite directions within \( \text{ponA} \) genes from strains Verdun and RZ11. This paper.

Protein expression of \( \text{pBPB} \) and \( \text{ponA} \) in E. coli. The \( \text{pBPB} \) gene from strain Verdun was amplified by PCR using the \( \text{pfuTurbo DNA polymerase} \) (Stratagene, La Jolla, Calif.) using primers PBPM3 and PBPSL (Table 2). Primers PBPM3 and PBPSL (1 µM each) were added to 10 ng of pG-PBP3 plasmid DNA. This allowed amplification of the \( \text{pBPB} \) sequence from 95 nucleotides after the start codon to the stop codon (which corresponded to the periplasmic predicted part of the protein). The 1,737-bp PCR product was cloned into pCR2.1 vector.
## TABLE 2. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>4(^a)</td>
<td>CATTAAAATCCCGATAGC</td>
<td>+651 to +634 downstream of the (pbpB) start codon (strain Verdun)</td>
</tr>
<tr>
<td>8(^a)</td>
<td>GAGTCATCTTCCTCAGTG</td>
<td>+88 to +105 downstream of the (pbpB) start codon (strain Verdun)</td>
</tr>
<tr>
<td>11(^a)</td>
<td>TAGTTTTGAATGAGTC</td>
<td>−76 to −59 upstream of the (pbpB) start codon (strain Verdun)</td>
</tr>
<tr>
<td>36(^a)</td>
<td>CGGTATCTCGGATTAT</td>
<td>−389 to −372 upstream of the (pbpB) start codon (strain Verdun)</td>
</tr>
<tr>
<td>12(^a)</td>
<td>CTCCCGGAATCTCCAGTG</td>
<td>−727 to −710 upstream of the (pbpB) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>29(^a)</td>
<td>CGAAGATCCGAAGGCTC</td>
<td>−92 to −109 upstream of the (pbpB) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>38(^a)</td>
<td>AGGCCGAACCTCTGAGAAA</td>
<td>+198 to +181 downstream of the (pbpB) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>39(^a)</td>
<td>TCTAAGTCTACCGGATTC</td>
<td>+520 to +503 downstream of the (pbpB) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>3(^b)</td>
<td>AGCGGCTTCTTGTTTATC</td>
<td>+1091 to +1074 downstream of the (ponA) start codon (strain Verdun)</td>
</tr>
<tr>
<td>Z157(^b)</td>
<td>GTCAGTCAAATTCGTCCA</td>
<td>+219 to +236 downstream of the (ponA) start codon (strain Verdun)</td>
</tr>
<tr>
<td>15(^b)</td>
<td>CTATTCTACATACAAGG</td>
<td>−390 to −373 upstream of the (ponA) start codon (strain Verdun)</td>
</tr>
<tr>
<td>7(^b)</td>
<td>GCCGTGAGAAGAAGATC</td>
<td>−600 to −583 upstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>Z195(^b)</td>
<td>TCTCCATCTTCTGCTTGA</td>
<td>−53 to −70 upstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>12(^b)</td>
<td>TGGCGGAAACAGGGCGCA</td>
<td>+193 to +176 downstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>17(^b)</td>
<td>CAAAATCATTCTCAATAAG</td>
<td>+498 to +481 downstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>php(\text{beg})(^c)</td>
<td>GAGGCCGGCTCCAATGGATTTGAA</td>
<td>+38 to +15 downstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>php(\text{end})(^c)</td>
<td>CCAGAAACTACAGATAATCCTCCA</td>
<td>−27 to −4 upstream of the (ponA) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>php(\text{beg})(^c)</td>
<td>TCTGAGTCTAGTTTTACGGGAAT</td>
<td>+27 to +43 downstream of the (pbpB) start codon (strain Verdun)</td>
</tr>
<tr>
<td>php(\text{end})(^c)</td>
<td>AGCGGGTTTTGTTACGAGCAGGAA</td>
<td>−75 to −52 upstream of the (pbpB) stop codon (strain Verdun)</td>
</tr>
<tr>
<td>oligo5.4(\text{UP})(^c)</td>
<td>GAGTTCACATGGATTTCAACTTG</td>
<td>Strain Verdun</td>
</tr>
<tr>
<td>oligo5.4(\text{RP})(^c)</td>
<td>GACGCCGTCATCTGGTTTATGTTG</td>
<td>Strain Verdun</td>
</tr>
<tr>
<td>PBP3(\text{L})(^d)</td>
<td>GGGGGGGGACCGCTCTCGG</td>
<td>+95 to +124 downstream of the (pbpB) start codon ((BamHI)) (strain Verdun)</td>
</tr>
<tr>
<td>PBP3(\text{M})(^d)</td>
<td>GGGGGGGGATCCGCTCTCTCTCGG</td>
<td>−30 to −1 upstream of the (pbpB) stop codon ((XhoI)) (strain Verdun)</td>
</tr>
<tr>
<td>121(^d)</td>
<td>AGTTTCAATCGGAGACAATTC</td>
<td>+1661 to +1642 of (ponA) (strain RZ11)</td>
</tr>
<tr>
<td>173(^d)</td>
<td>GATCAACACAAATCGCAGTCA</td>
<td>+1500 to +1519 of (ponA) for genome walking (strain RZ11)</td>
</tr>
<tr>
<td>184(^d)</td>
<td>CTGAAAGCCTATGTTTACAGTG</td>
<td>84 bp downstream of (ponA) (strain RZ11)</td>
</tr>
<tr>
<td>185(^d)</td>
<td>TCTGATTTCTTCTCCC</td>
<td>+467 to +448 of (pbpB) (strain RZ11)</td>
</tr>
<tr>
<td>186(^d)</td>
<td>GCATGTTTCTTCTCCC</td>
<td>+681 to +700 of (pbpB) (strain RZ11)</td>
</tr>
<tr>
<td>921</td>
<td>CGCGTTTGAGAAGAGAA</td>
<td>−19 to +1 of (pbpB)</td>
</tr>
<tr>
<td>922</td>
<td>CGAAGCTCTCAGAATAGAA</td>
<td>173 bp downstream of the (pbpB) stop codon</td>
</tr>
<tr>
<td>155</td>
<td>GCCGTGTTTGATTCGTTCC</td>
<td>Starts 232 bp upstream of (ponA) (strain RZ11)</td>
</tr>
<tr>
<td>513</td>
<td>TTGAAATGAGGAGACGAGAATA</td>
<td>Starts 389 bp downstream of (ponA) (strain RZ11)</td>
</tr>
</tbody>
</table>

\(^a\) Primers used for \(pbpB\) RT-PCR.

\(^b\) Primers used for \(ponA\) RT-PCR.

\(^c\) Primers used for the LD-PCR.

\(^d\) \(BamHI\) and \(XhoI\) sites are underlined.
**RESULTS AND DISCUSSION**

**Characterization of the L. interrogans pbpB gene.** Partial sequence analysis of a 1.5-kb fragment of *L. interrogans* serovar icterohaemorrhagiae strain Verdun showed that it contained part of a gene similar to those encoding bacterial HMW PBPs (4). This fragment was used to screen a cosmid library of strain Verdun genomic DNA by colony hybridization to isolate a complete copy of the gene and surrounding DNA. The region surrounding this putative PBP gene was subcloned and sequenced. One open reading frame (ORF) with the potential to encode a 602-amino-acid protein, having an estimated molecular mass of 67.3 kDa according to the compute pI/Mw tool (23), was identified. The protein sequence deduced from this ORF was used to search the GenBank and EMBL databases for homologs using BLASTP. This protein was most similar to several HMW PBPs, including the *Bacillus subtilis* stage V sporulation protein D; PBP 1 and PBP 3 of *Borrelia burgdorferi* and *Treponema pallidum*, respectively; cell division protein FtsI of *Streptomyces coelicolor*; PBP A2 of *Rickettsia prowazekii*; and PBP 3 of *E. coli*. Pairwise comparison revealed that *L. interrogans* protein shares about 30 and 26% sequence identity with the PBP 3 proteins from *T. pallidum* and *E. coli*, respectively. Because of the strong similarity to the gene encoding PBP 3, this gene was designated *pbpB*. 

**Identification of PBPs by labeling with DIG-AMP.** Pellets of *E. coli* harboring plasmids containing the *pbpB* and *ponA* genes and their vector controls were suspended in phosphate-buffered saline and sonicated. Aliquots of the sonicated cells (100 μg of protein) were incubated at 37°C for 10 min with 2.5 μg of AMP per ml conjugated to digoxigenin (DIG) as described by Weigel et al. (22). Of each sample, 12.5 μg was resolved by SDS-PAGE; PBPs were identified by immunoblotting with an anti-DIG–alkaline phosphatase conjugate (Boehringer Mannheim Corp., Indianapolis, Ind.) followed by chemiluminescence from CDP Star (Boehringer Mannheim Corp.). In all competition experiments, samples were incubated for 30 min with a 400-fold excess of free AMP (Sigma Chemical Co.).

**Nucleotide sequence accession number.** The *pbpB* and *ponA* sequences from strain Verdun have been assigned the EMBL accession no. AJ243720 and AJ278610, respectively. The *ponA* and *pbpB* sequences from strain RZ11 have been assigned the EMBL accession no. AF262906 and AF262907, respectively.

**RESULTS AND DISCUSSION**

Characterization of the *L. interrogans* *pbpB* gene. Partial sequence analysis of a 1.5-kb fragment of *L. interrogans* serovar icterohaemorrhagiae strain Verdun showed that it contained part of a gene similar to those encoding bacterial HMW PBPs (4). This fragment was used to screen a cosmid library of strain Verdun genomic DNA by colony hybridization to isolate a complete copy of the gene and surrounding DNA. The region surrounding this putative PBP gene was subcloned and sequenced. One open reading frame (ORF) with the potential to encode a 602-amino-acid protein, having an estimated molecular mass of 67.3 kDa according to the compute pi/Mw tool (23), was identified. The protein sequence deduced from this ORF was used to search the GenBank and EMBL databases for homologs using BLASTP. This protein was most similar to several HMW PBPs, including the *Bacillus subtilis* stage V sporulation protein D; PBP 1 and PBP 3 of *Borrelia burgdorferi* and *Treponema pallidum*, respectively; cell division protein FtsI of *Streptomyces coelicolor*; PBP A2 of *Rickettsia prowazekii*; and PBP 3 of *E. coli*. Pairwise comparison revealed that *L. interrogans* protein shares about 30 and 26% sequence identity with the PBP 3 proteins from *T. pallidum* and *E. coli*, respectively. Because of the strong similarity to the gene encoding PBP 3, this gene was designated *pbpB*.
To determine the level of genetic drift between the genetically similar but distinct serovars icterohaemorrhagiae and pomona, the corresponding \( pbpB \) gene of strain RZ11 (serovar pomona) was amplified, cloned, and sequenced. The two \( L.\ interrogans \) \( pbpB \) sequences are 99% identical, with 13 base mismatches over a 2,409-bp ORF. Analysis of the derived proteins from both genes revealed that all but two of the sequence changes were silent. The amino acid changes detected were Met435 to Thr435 and Glu468 to Gly468 (changes are written as Verdun to RZ11). The deduced \( pbpB \) 3 proteins from both strains had eight sequence motifs that are well conserved among class B PBPs (Table 3). Three motifs found in the C-terminal domain are also common to penicilloytransferases (8). The active-site serine residue that binds to penicillin is typically part of the motif SXXK (box 6), and this was located at residue 259 in \( pbpB \) 3. The SXN and KTG motifs present in the active site of every penicillin-binding domain were located at residues 312 and 465 (boxes 7 and 8, respectively). The spacing between these active-site motifs was well conserved, as was the spacing between the other regions of similarity (Table 3).

**Characterization of the \( L.\ interrogans \) \( ponA \) gene.** A plasmid clone, pKB1, containing a portion of the strain RZ11 \( ponA \) gene, encoding \( PBP1 \), was identified during a study using sequence analysis of randomly selected clones to improve resolution of the combined physical and genetic map of \( L.\ interrogans \) (4). Plasmid pKB1 contains about two-thirds of the gene, including the 5’ end. A genomic walking technique was used to amplify the 3’ end of the gene using primer 173. The resulting 1,300-bp amplicon was cloned, generating plasmid pK127, and sequenced. The overlapping sequences of pKB1 and pK127 revealed the presence of a 2,409-bp ORF, with the potential to encode an 802-amino-acid protein with a predicted mass of 89.8 kDa according to the compute pl/Ms tool (23). The deduced protein was used to search the GenBank database using BLASTP. This sequence was most similar to those of \( N.\ gonorrhoeae \) and \( E.\ coli \) \( pbpB \) 1 and 1a, respectively. The \( L.\ interrogans \) gene was designated \( ponA \) because of its similarity to the \( E.\ coli \) \( ponA \) gene. A cosmid containing the strain Verdun \( ponA \) gene was identified by colony hybridization using a 1-kb \( ClaI \) fragment derived from pKB1 as a probe.

The two \( L.\ interrogans \) \( ponA \) sequences are 98% identical with 30 base mismatches over a 2,409-bp ORF. Analysis of the derived proteins from both genes revealed that there are 19 silent mutations, 5 conserved mutations, and 6 nonconserved mutations. Interestingly, most of the mutations occur in the amino-terminal portion of the sequence. There is one nonconservative mutation in the putative transmembrane helix (Thr to Ile).

Further evidence that the \( L.\ interrogans \) \( ponA \) gene encoded an HMW PBP was gained by identification of consensus motifs common to class A HMW PBPs. \( pbpB \) 1 from both strains, Verdun and RZ11, contain the nine boxes that are conserved in all PBPs of this class (Table 4). Furthermore, each of the three consensus motifs of the active site was identified in the deduced amino acid sequence of \( ponA \), and the intervals between these motifs were consistent with those of other PBPs.

**The \( pbpB \) and \( ponA \) genes are 8 to 10 kb apart and comprise individual transcription units.** The cloned \( ponA \) and \( pbpB \) genes were previously localized on the \( L.\ interrogans \) strain RZ11 and Verdun physical maps by hybridization (4). These data showed that the two genes were located in the same region of the genome. However, the methodology used for mapping lacks detailed resolution, and thus, it could not be determined if these two genes were closely linked in the genome. The approximate distance between \( pbpB \) and \( ponA \) was determined using LD-PCR. The primers used for this analysis were located at the beginning and end of both genes and were directed outward toward flanking sequences (Table 2). Initial LD-PCR results showed that the two genes were about 8 kb apart (strain Verdun) and 10 kb apart for strain RZ11. Further, primers for \( ponA \) and \( pbpB \) were confirmed for both strains. For strain Verdun, using additional primers that anneal to a 5.4-kb \( XbaI \) fragment found between \( ponA \) and \( pbpB \) genes, the 8.5-kb distance between these two genes was confirmed. For strain RZ11, the 10.4-kb distance was confirmed, indicating that there may be a small insertion between \( ponA \) and \( pbpB \) in strain RZ11 compared to strain Verdun.

The transcription of both genes from strain Verdun was analyzed by RT-PCR. For the \( pbpB \) gene, internal primers allowed reverse transcription of RNA, indicating that \( pbpB \) is transcribed (Fig. 1A, lanes 1 and 7). Primers close upstream and downstream of the gene in use with internal primers still allow reverse transcription (Fig. 1A, lanes 3 and 9), while primers located further upstream and downstream of the ORF

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**Table 4.** Boxes conserved between the PBPs 1 from both the Verdun and RZ11 strains of \( L.\ interrogans \) and other related PBPs

<table>
<thead>
<tr>
<th>PBP (species)</th>
<th>Box 1</th>
<th>Spacing</th>
<th>Box 2</th>
<th>Spacing</th>
<th>Box 3</th>
<th>Spacing</th>
<th>Box 4</th>
<th>Spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDX(R/K)F/EXH/G</td>
<td>120</td>
<td>20</td>
<td>150</td>
<td>14</td>
<td>172</td>
<td>12</td>
<td>189</td>
<td>55</td>
</tr>
<tr>
<td>PBP 1 (( L.\ interrogans ))</td>
<td>EDYEFYNHIG</td>
<td>21</td>
<td>144</td>
<td>14</td>
<td>166</td>
<td>12</td>
<td>183</td>
<td>55</td>
</tr>
<tr>
<td>PBP 1b (( S.\ sp. ))</td>
<td>EDYEFYNHIG</td>
<td>21</td>
<td>119</td>
<td>14</td>
<td>141</td>
<td>12</td>
<td>158</td>
<td>55</td>
</tr>
<tr>
<td>PBP 1 (( N.\ gonorrhoeae ))</td>
<td>EDYEFYNHIG</td>
<td>21</td>
<td>117</td>
<td>14</td>
<td>139</td>
<td>12</td>
<td>156</td>
<td>55</td>
</tr>
<tr>
<td>PBP 1a (( E.\ coli ))</td>
<td>EDYEFYNHIG</td>
<td>21</td>
<td>117</td>
<td>14</td>
<td>139</td>
<td>12</td>
<td>156</td>
<td>55</td>
</tr>
</tbody>
</table>

* \( pbpB \) 1 sequences from \( L.\ interrogans \) strains Verdun and RZ11 have the same conserved boxes. Numbers above the sequences indicate the positions. Spacing indicates the number of amino acids between boxes. Amino acids conserved between PBPs are indicated in boldface; where they are not conserved, they are underlined.
in use with internal primers do not (Fig. 1A, lanes 5 and 11). The start of transcription can thus be located between 76 and 389 bp upstream of the \( \text{pbpB} \) gene. Analogously, transcription of the \( \text{ponA} \) gene was demonstrated (Fig. 1B, lanes 1, 5, and 7). Amplicons were not formed when RT was absent from the reaction mix (Fig. 1A and B, lanes with even numbers). Taken together, the results (Fig. 1C) indicate that both genes are transcribed as single transcription units. Analysis of the strain RZ11 \( \text{ponA} \) gene confirmed that it was also transcribed (data not shown).

**PBP 1 and PBP 3 bind AMP.** The \( \text{L. interrogans} \) PBP 1 and PBP 3 proteins resemble other HMW PBPs, and both proteins retain signature motifs associated with penicillin binding sites (14). Based on these similarities, we predicted that both proteins would covalently bind penicillin. As a first step, we wished to compare the sizes of PBPs in \( \text{L. interrogans} \) to those of the five PBPs identified for \( \text{L. kirschneri} \) (10). Differences were detected with the PBP 1 and 2 proteins. As a second step, we wished to compare the masses of PBPs in \( \text{L. interrogans} \) to those of other bacteria. Further analysis of the transmembrane-spanning and anchoring functions of these proteins may provide insight into the mechanism of protein secretion. Ongoing work in this laboratory is directed at determining the structure of the \( \text{L. interrogans} \) cell envelope organization of spirochetes is unique, having features in common with both gram-positive and gram-negative bacteria. For example, the spirochetal cytoplasmic membrane is intimately associated with the peptidoglycan cell wall, as it is in gram-positive bacteria. Like gram-negative bacteria, spirochetes have an outer membrane, but this membrane is unusual, being the most fluid membrane known to exist in nature (5). These differences may influence the mechanisms of protein secretion. The process of protein secretion is poorly characterized for spirochetes. However, Haake (9) has recently identified a lipoprotein anchor consensus sequence shared by spirochetes that is slightly different from that of other bacteria. Further characterization of the signal sequences for the PBPs should...
provide insight into the mechanisms by which spirochetal proteins are secreted and the signals used for membrane insertion. These signal sequences may also be useful in targeting leptospiral proteins to the E. coli periplasm to assist in purification.

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REFERENCES

Girons. 1997. Borelia burgdorferi uridine kinase: an enzyme of the pyrimi-
dine salvage pathway for endogenous use of nucleotides. FEMS Microbiol.
body-coated latex beads attached to the spirochete Leptospira interrogans.
pomona and growth of 13 other serotypes: fractionation of oleic albumin
26:45–51.
7. Faine, S., B. Adler, C. Bolin, and P. Perolat. 1999. Leptospira and leppto-
riosis. Medici, Melbourne, Australia.
M. A. Lovett. 1991. Changes in the surface of Leptospira interrogans serovar
12. Johnson, R. C., and V. G. Harris. 1967. Differentiation of pathogenic and
31.
penicillin-binding proteins and beta-lactamases. Antimicrob. Agents Che-
The structure and function of Escherichia coli penicillin-binding protein 3.
Pseudomonas aeruginosa: involvement in adaptive and mutational resistance
susceptibilities of five Leptospira strains to 16 antimicrobial agents. Antimi-
17. Piras, G., D. Rare, A. El Kharrouchi, D. Hastir, S. Englebert, J. Coyette, and
J. Ghuysen. 1993. Cloning and sequencing of the low-affinity penicillin-
binding protein S3r-encoding gene of Enterococcus hirae S185: modular design
laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, N.Y.
and P. J. Ketterer. 1997. Amoxycillin as an alternative to dihydrostreptomycin
salt for treating cattle infected with Leptospira borgpeterseni serovar
20. Spratt, B. G. 1977. Properties of the penicillin-binding proteins of Esche-
a proposal based on their structural similarity to acyl-D alanly-D-alanine.
genin-ampicillin conjugate for detection of penicillin-binding proteins by
Appel, and D. F. Hochstrasser. 1999. Protein identification and analysis tools
comprising the genome of Leptospira interrogans. Nucleic Acids Res. 19:4857–
4860.
Characterization of the Leptospira interrogans S10-spc-alpha operon. FEMS