Cloning of the Major Outer Membrane Protein Expression Locus in *Anaplasma platys* and Seroreactivity of a Species-Specific Antigen

Tzung-Huei Lai,¹ Nelson G. Orellana,² Yumi Yuasa,³ and Yasuko Rikihisa¹*

Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210-1093; Departamento de Medicina Cirúrgica, Decanato de Ciencias Veterinarias, Universidad Centroccidental Lisandro Alvarado, Tarabana, Estado Lara, Venezuela; and Department of Veterinary Medicine, National Chung Hsing University, Taichung, 402 Taiwan, Republic of China

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*Corresponding author. Mailing address: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210-1093. Phone: (614) 292-5661. Fax: (614) 292-6473. E-mail: rikihisa.1@osu.edu.

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Anaplasma platys (formerly known as *Ehrlichia platys*) was first identified in 1978 in Florida as a *Rickettsia*-like bacterium found in the platelets of dogs with infectious canine cyclic thrombocytopenia (ICCT) (26). The original description noted the morphological and biological similarity of this bacterium to *Ehrlichia canis* in infected dogs and to *Anaplasma marginale* in infected cattle, both of which are current members of the family *Anaplasmataceae* (17, 26). Indirect fluorescent antibody testing of platelet-rich plasma from dogs experimentally infected with ICCT showed minimal serological cross-reactivity between the new bacterial species and *A. canis*; therefore, a new bacterial species was proposed and named “*Ehrlichia platys*” (23). In 1992, Anderson et al. (3) reported the 16S rRNA gene sequence of *A. platys* in Louisiana. Subsequently, the groEL gene sequence of *A. platys* was also determined (29, 67). Phylogenetic analysis of these two gene sequences showed that the new bacterial species was distinct from but closely related to *Anaplasma phagocytophilum* and *A. marginale*, which led to reclassification of the new bacterial species into the genus *Anaplasma* (17). *A. platys* infections in the platelets from a naturally infected dog were shown to cross-react with mouse anti-*A. phagocytophilum* serum (32).

Clinical signs of ICCT are fever, depression, and anorexia (23). Parasitemia and thrombocytopenia occur in cycles of approximately 10 to 14 days (23). In the United States, dogs that are seropositive for *A. platys* have been found in Florida, Louisiana, Mississippi, Texas, Arkansas, North Carolina, Pennsylvania, Illinois, Idaho, and California, and dogs were frequently seropositive for both *A. platys* and *E. canis* (23). Internationally, *A. platys* DNA has been detected in blood of dogs from Brazil (21), Greece (43), France (33), Spain (54), Portugal (13), Taiwan (15), China (28), Japan (61), Thailand and Venezuela (29, 59), Australia (12), and the Republic of the Congo (55). *A. platys* has been found in the brown dog tick *Rhipicephalus sanguineus* in Japan (34), Spain (58), and the Republic of the Congo (55); however, it has not been proven whether *R. sanguineus* is a biological vector of *A. platys* (56). *A. platys* has not been isolated in culture, and the genes, proteins, and antigens of *A. platys* are not known.

In *A. phagocytophilum* and *A. marginale*, surface-exposed, immunodominant, 44-kDa major outer membrane proteins (OMPs) (*P44s/Msp2s*) are encoded by the *p44/msp2* polymorphic multigene family (6, 9, 39, 41, 69–71). In *A. phagocytophilum*, *P44* proteins consist of a single central hypervariable region of approximately 94 amino acids (aa) and N-terminal and C-terminal conserved regions of approximately 186 and 146 amino acids, respectively (41). A single polymorphic *p44/msp2* expression locus (*p44/msp2ES*) is found in the genome of *A. phagocytophilum* and *A. marginale* (10, 18). In both species, this expression locus is found downstream from the *tr1/tr2* gene, which encodes a putative transcription factor, and homologs of *Ehrlichia omp-1, p28/p30, map-1* genes, which encode immunodominant polymorphic major OMPs (6, 8, 39). At the *p44/msp2ES* locus, *p44s* and *msp2* donor sequences from elsewhere...
FIG. 1. Strategy for *A. platys* (A. *pl*) major outer membrane protein expression locus sequencing. (A) *A. phagocytophilum* (A. *ph*) *p44ES* and *A. marginale* (A. *ma*) *msp2ES* were aligned to design primer F1 (targeting the highly conserved region upstream of tr1/tr) and the degenerate primers R1 (targeting the *p44ES/msp2 C*-terminal region), R2 (targeting the conserved intergenic region between *omp-1X omp1* and *omp1-N*/*opag3*), R3 (targeting the *p44ES/msp2 N*-terminal region), and R4 (targeting the conserved *valS* gene downstream of *p44ES/msp2ES*). Primers F2 and F3, indicated in panel B, were designed based on the sequence results. (B) The final sequence (3,957 bp) was assembled using the SeqMan program within the DNASTAR software. Genes are represented as boxes with arrows indicating their orientation. Numbers indicate base pairs. (C) The entire expression locus fragment D (arrowhead) amplified from the dog 2 blood DNA specimen by primers F1 and R5.

in the genome undergo gene conversion via a RecF-dependent pathway, allowing the expression of various *p44* donor sequences at this locus via a single promoter (6, 8, 39, 40). This mechanism is thought to facilitate F44/Msp2 antigenic variation during acute and persistent infection and to facilitate adaptation to new environments, such as during transmission between tick and mammalian hosts (7, 11, 38, 40, 65, 71). Purified native P44 from *A. phagocytophilum* and purified native OMP-1s (P28 and OMP-1F) of *Ehrlichia chaffeensis* have porin activity (30, 37).

In the present study, we first isolated a major outer membrane protein expression locus in *A. platys*. Second, we compared the major outer membrane protein expression loci among *A. platys*, *A. marginale*, and *A. phagocytophilum*. Third, we analyzed the structure of major outer membrane proteins of *A. platys* using bioinformatics tools. Fourth, we determined *A. platys*-specific amino acid sequences predicted to be antigenic and located in the external loop regions of β-barrel proteins. Finally, we tested the immunoreactivity of one peptide by enzyme-linked immunosorbent assay (ELISA) using *A. platys*-positive canine serum. The results suggest the potential to use these peptides for serodiagnosis of *A. platys* infection.

**MATERIALS AND METHODS**

*A. platys*-infected dogs. Dogs that were naturally infected with *A. platys* were identified in Lara, Venezuela, in 2007 by observation of bacterial inclusions (morulae) in platelets from blood smears, and cases were confirmed by PCR and sequencing using primer pairs specific for *A. platys* 16S rRNA (EP1-EP3 and EP2-EP3) (29). Naturally infected dogs in Taichung, Taiwan, Republic of China, were identified in 2010 and confirmed by PCR using the primer pair EPLATS-EPLAT3 (42).

Cloning of *p44* expression locus from *A. platys*. DNA samples from three dogs from Venezuela and one dog from Taiwan were used as templates. By aligning the *p44*/*msp2* expression loci from *A. phagocytophilum* and *A. marginale*, we were able to design several degenerate primers for conserved regions of the locus (Fig. 1; primers are available upon request). Using the first and the second primer pairs, F1-R1 and F1-R2, (hemi-)nested touchdown PCR (52) was used to amplify the tr1 and omp-1X gene sequences from *A. platys*. In order to avoid truncating *p44* pseudogenes in the *A. platys* genome, we designed primer F3 upstream of the predicted *p44* open reading frame. *p44ES* sequences were amplified by nested touchdown PCR using primer pairs F2-R3 and F3-R4. Amplification was performed as previously described (68). The amplified DNA fragments were cloned using a TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced with M13 forward or M13 reverse sequencing primers. All sequencing data were assembled using the SeqMan program (DNASTAR, Inc., Madison, WI). To confirm the assembly, the entire locus was amplified using primers F1 and R5 (primers are available upon request).

Phylogenetic analysis. The deduced amino acid sequences for Tr1, OMP-1X, OMP-1X, and P44ES from *A. marginale*, *A. phagocytophilum*, and *A. platys* were aligned using the MegaAlign program (DNASTAR, Inc.) by the Chastul W method.

Protein structure analysis using bioinformatics tools. The SignalP 3.0 server trained on Gram-negative bacteria (http://www.cbs.dtu.dk/services/SignalP) was used for signal peptide sequence analysis. The secondary structures of *p44* and OMP-1X were predicted by PRED-TMBB (4) and hydrophobicity analysis and the hydrophobic moment profile method, as previously described (30, 35). The antigenic index and surface probability were determined using the Protean program (DNASTAR, Inc.).

ELISA analysis of OMP-1X-specific peptide. The OMP-1X peptide from *A. platys* was synthesized at Biomatik (Wilmington, DE). The purity of the peptide was greater than 98%, as assessed by high-performance liquid chromatography. The wells of a 96-well microtiter plate were coated with 200 ng peptide/well, and the ELISA was performed as previously described (60). Samples were from three dogs that were PCR positive for *A. platys* (TW 431, TW 270, and TW 210) and three dogs that were both PCR negative for *A. platys* and antigen dot blot negative for *A. phagocytophilum* (E05-290, E10-0062, and E10-0075). In addition, horse anti-*A. phagocytophilum*-positive sera (EQ002, EQ006, and EQ09-0011) (65, 71) were used to confirm the absence of OMP-1X peptide antigen cross-reactivity with anti-*A. phagocytophilum* antibodies. The horseradish peroxidase substrate 2,2′-azido-di-(3-ethyl)-benzthiazoline-6-sulfonic acid (Sigma, St. Louis, MO) in 70 mM citrate buffer (pH 4.2) was applied, and absorbance values at 415 and 492 nm were measured in an ELISA plate reader (Molecular Devices, Sunnyvale, CA) as previously described (68). The results are presented as the optical density at 415 nm minus that at 492 nm (OD415 - OD492), and the cutoff for a positive reaction was set at greater than the mean of the value for the formula OD415 - OD492 + 3 standard deviations (SD) for the negative-control samples (OD > 0.165). The assay was repeated at least three times.

Nucleotide sequence accession numbers. The *A. platys* tr1-omp-1X-p44ES sequences from two naturally infected dogs from Venezuela were assembled and
RESULTS

Cloning of the *A. platys* outer membrane protein expression locus. We designed three degenerate primers and one primer at the highly conserved upstream region of *tr1* based on alignment of the *tr1-omp1X-omp1N-p44* gene cluster from *A. phagocytophilum* (GenBank sequence accession number AY137510) with the *tr-omp1-opag3-opag2-opag1-msp2* gene cluster from *A. marginale* (GenBank sequence accession number AY132308) (Fig. 1, primers are available upon request). The first touchdown PCR (52) was designed to amplify the entire cluster fragment using primers F1 and R1 (Fig. 1) (primers are available upon request). The PCR products were then used as templates for heminested touchdown PCR using primers F3 and R3 (Fig. 1) (primers are available upon request). A single band, approximately 2,100 bp in size, was amplified (fragment A). The PCR product was cloned using a TA cloning kit and sequencing showed that fragment A contained *A. platys* tr1 and a homolog of *omp-IX* (named here *A. platys omp-1X*).

The region downstream from fragment A was amplified by nested touchdown PCR using the PCR products obtained with primers F1 and R1 as a template, with primer F2 based on fragment A and primer R3 based on conserved sequences for *p44* (*msp2*) in *A. phagocytophilum* and *A. marginale*. A single band, approximately 1,100 bp in size, was amplified (fragment B). The PCR product was cloned using the TA cloning kit, and sequencing showed that fragment B contained a partial sequence for *A. platys* p44ES. To amplify the full-length p44ES from *A. platys*, primer F3 was designed based on fragment B and primer R4 was designed based on the conserved region of *valS* found downstream from *p44* (*msp2*) in *A. phagocytophilum* and *A. marginale*. Another touchdown PCR was conducted, using primers F1 and R4. The PCR products were then used as templates for heminested touchdown PCR using primers F3 and R4. A single band, approximately 1,700 bp in size, was amplified (fragment C). The PCR products were cloned using the TA cloning kit, and sequencing showed that fragment C contained the full-length p44 sequence from *A. platys*.

The final assembled sequence of 3,957 bp from Venezuelan dogs 1 and 2 contained the entire *A. platys* p44ES locus. The average sequence coverage of the entire locus is 8.3-fold (5- to 15-fold). To confirm that the assembly is from a complete genomic locus, we designed primer R5 downstream of the predicted *p44* open reading frame and conducted one more touchdown PCR using primers F1 and R5. A single band of approximately 3.9 kb was amplified (fragment D), indicating that the fragment containing the entire locus was amplified from the blood of dog 2 (Fig. 1C). The G+C content was determined to be 47.46% to 47.51%. The synteny among the entire outer membrane protein gene clusters of *A. platys* and the two previously sequenced *Anaplasma* species was analyzed using the Artemis Comparison Tool (14). The *tr1* sequences were conserved among the three *Anaplasma* species (Fig. 2). The 5′ and 3′ regions of *p44ES* were conserved between *A. platys* and *A. phagocytophilum* but less conserved between *A. platys* and *A. marginale* (Fig. 2).

**A. platys** *Tr1* structure. Three similar (97.8% to 99.5%) *A. platys* tr1 sequences were obtained from two dogs from Venezuela and one dog from Taiwan. The predicted molecular mass of *A. platys* Tr1 was 21.0 to 21.1 kDa, and the isoelectric point was 5.50 to 5.80 (Table 1). Tr1 was not predicted to have a signal peptide and, thus, is a cytoplasmic protein, as analyzed by SignalP 3.0. Tr1 was predicted to contain a putative N-terminal helix-turn-helix DNA-binding domain, based on the analysis of the NCBI conserved domain database, suggesting that it is a transcriptional regulator. The amino acid sequence identity between *A. platys* Tr1 and *A. phagocytophilum* Tr1 (GenBank sequence accession number YP_505749) was 84.8% to 86.4%, and that between *A. platys* Tr1 and *A. marginale* Tr (GenBank sequence accession number YP_154239) was 73.1% to 74.1%.

**A. platys** *OMP-1X* structure. Three nearly identical (99.1%) *A. platys* omp-1X sequences were obtained from two dogs from Venezuela and one dog from Taiwan. Using the SignalP 3.0

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**TABLE 1. Properties of the *A. platys* p44ES cluster**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Upstream intergenic space (bp)</th>
<th>Gene length (bp)</th>
<th>Protein size (amino acids)</th>
<th>Signal peptide (amino acids)</th>
<th>Molecular mass (Da)</th>
<th>Isoelectric point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tr1</td>
<td>NA*</td>
<td>558</td>
<td>185</td>
<td>NA</td>
<td>21010.6–20952.6</td>
<td>5.50–5.80</td>
</tr>
<tr>
<td>OMP-1X</td>
<td>306</td>
<td>933</td>
<td>301</td>
<td>23</td>
<td>31885.0–31942.1</td>
<td>7.27–7.92</td>
</tr>
<tr>
<td>P44ES</td>
<td>682</td>
<td>1,221</td>
<td>380–386</td>
<td>21</td>
<td>41167.3–41359.5</td>
<td>5.30–5.72</td>
</tr>
</tbody>
</table>

* Mature protein.
* Predicted cleavage site.
* NA, not applicable.
* Range among strains or alleles.
server, OMP-1X was predicted to have a signal peptide with a cleavage site between positions 23 and 24. The predicted molecular mass of mature *A. platys* OMP-1X was 31.9 kDa and the isoelectric point 7.27 to 7.92 (Table 1). We then examined the secondary structure of OMP-1X, using PRED-TMBB (4). The discrimination value of the OMP-1X amino acid sequence was 2.907, which is below the threshold value of 2.965, making OMP-1X likely to be a β-barrel protein localized to the outer membrane. Hydrophobicity analysis and the hydrophobic moment profile program, developed for porin structure prediction (15), predicted 14 β-strands in OMP-1X. The protein sequences most closely related to *A. platys* OMP-1X are *A. phagocytophilum* OMP-1X (GenBank sequence accession number YP_505750; 45.9%-46.3% identity) and *A. marginale* OMP-1 (GenBank sequence accession number YP_154240; 39.8% identity). A phylogenetic analysis showed that OMP-1X homologs in *Anaplasma* spp. form a cluster that is distinct from the cluster of most closely related OMP-1X homologs in each *Ehrlichia* spp. (Fig. 3).

*A. platys* P44ES structure. Four P44ES sequences (GenBank sequence accession numbers GQ868750, GU357491, GU357492, and GU357493) were obtained from three dogs from Venezuela. Using the SignalP 3.0 server, P44ES was predicted to have a putative signal peptide with a cleavage site between positions 21 and 22. The predicted molecular mass of mature P44ES protein was predicted to be 41.2 to 41.4 kDa and the isoelectric point 5.30 to 5.72 (Table 1). By PRED-TMBB (4) analysis, the discrimination value of the P44 amino acid sequence was 2.920, which is below the threshold value of 2.965, making P44 likely to be a β-barrel protein localized to the outer membrane. Hydrophobicity analysis and the hydrophobic moment profile predicted 16 β-strands in P44. Alignment of a total of nine *A. platys* P44 sequences (the four P44 full-length proteins from dogs 1, 2, and 3 and the five partial P44 sequences obtained from dogs 1, 2, and Taiwan) using the HVF and HVR primers revealed a single central hypervariable region (aa position 193 to 247) of approximately 55 amino acid residues and N-terminal and C-terminal conserved regions of approximately 192 and 159 amino acid residues, respectively.

The conserved amino acids C, C, W, and A from the P44 hypervariable region of *A. phagocytophilum* P44 (41) were also detected in the hypervariable region of *A. platys* P44. The C terminus of *A. platys* P44 ends with phenylalanine, as does the C terminus of *A. phagocytophilum* P44 (30). The amino acid sequence identity between *A. platys* P44ES and *A. phagocytophilum* P44-18ES (GenBank sequence accession number YP_505752) was 55.0% to 56.9%, and that between *A. platys* P44ES and *A. marginale* Msp2 (GenBank sequence accession number YP_154245) was 41.5% to 42.1%. Phylogenetic analysis placed full-length *A. platys* p44s between *A. phagocytophilum* p44s and *A. marginale* msp2s (Fig. 4). The sequence identities of the conserved N-terminal 192 amino acids and the conserved C-terminal 159 amino acids of *A. platys* and *A. phagocytophilum* P44s were 57.3% and 66.7%, respectively.

Primer pairs (HVF and HVR; primers are available upon request) designed based on the *A. platys* p44 conserved region amplified only *A. platys* DNA and not *A. phagocytophilum* and *A. marginale* DNA (data not shown). Alignment of a total of nine *A. platys* P44 hypervariable regions and flanking conserved regions with P44/Msp2 sequences among *A. phagocytophilum* P44s and *A. marginale* Msp2s revealed several *A. platys*-specific sequences: TGTAAGSDVDYVSKF (aa position 23 to 37), TRVWVKAE (aa position 78 to 85), AAEEVKAAXAVG TSAK (aa position 174 to 189), SWKCTQTG (aa position 207 to 214), AAKAEDLS (aa position 248 to 255), and ATTNK TKEF (aa position 378 to 386). These *A. platys*-specific p44 regions could be utilized as serologic test antigens to distinguish *A. platys* infections from *A. phagocytophilum* or *A. marginale* infections.

**ELISA analysis of OMP-1X.** When the Clustal W method was used to compare *A. platys* OMP-1X to its phylogenetically closest OMP-1 homologs—*A. phagocytophilum* OMP-1X (YP_505750), *A. marginale*OMP1 (YP_154240), *E. canis* P30-19 (AAK28680), *E. ewingii* (YP_180721), *E. ruminantium* Map1-related protein (YP_154240),...
and *E. chaffeensis* OMP-1 M (YP_507903) (GenBank sequence accession numbers are in parentheses)—we identified a unique region in the *A. platys* OMP-1X amino acid sequence. This sequence, AVQEKPKPEA, is within the 2nd external loop from the N terminus based on the hydrophobicity analysis and the hydrophobic moment profile program. The sequence is predicted by the Protean program to be highly antigenic and surface exposed, which may aid in differential serodiagnosis (Fig. 5A). The *A. platys* OMP-1X peptide was synthesized, and its reactivity to known infected dog sera was tested by ELISA. Three *A. platys* PCR-positive dog sera reacted with the synthesized OMP-1X peptide antigen. Sera from *A. platys* PCR-negative dogs (bars 4 to 6), and *A. phagocytophilum*-seropositive dogs (bars 7 to 9), the y axis shows values for (OD$_{415} -$ OD$_{492}$) ± SD. A reaction was considered positive when the value was greater than the mean value for (OD$_{415} -$ OD$_{492}$) + 3 SD for negative-control plasma (dashed line). The data shown are representative of triplicate assays.

**DISCUSSION**

In the present study, the entire 4-kb *A. platys* major outer membrane protein expression locus, containing the tr1, omp-1X, and p44 genes, was sequenced, providing new insight into the p44 expression locus and major surface antigens of *A. platys*. Different p44ES sequences were detected from individual dogs infected with *A. platys*, suggesting that there is a mixed p44 allele population of *A. platys*, similar to observations for *A. phagocytophilum* p44 expression in humans, mice, and horses (38, 39, 65) and *A. marginale* msp2 expression in cattle (19, 22, 47). The p44 primer pair HVF and HVR, designed in this study, can be used to obtain a more complete *A. platys* repertoire of p44 sequences in various geographic regions in order to learn about P44 antigen diversity among *A. platys* strains. Future analysis should determine whether multiple copies of p44 are present in the *A. platys* genome, as is the case for *A. phagocytophilum* p44 and *A. marginale* msp2 (10, 18, 69); this would further contribute to understanding the p44/msp2 multigene family, which is characterized by highly active intragenomic recombination.

Our synteny analysis suggests that the major outer membrane expression locus existed in a common ancestor of the three *Anaplasma* species in existence today. Furthermore, the locus appears to have diverged primarily by duplicating omp-1-like sequences between tr1 and p44/msp2ES; *A. marginale*, *A. phagocytophilum*, and *A. platys* have 4, 2, and 1 omp-1-like sequences, respectively. The three species of *Anaplasma* infect different types of host cells, namely, erythrocytes, neutrophils, and platelets. Comparative studies of P44/Msp2s and OMP-1 homologs from *A. marginale*, *A. phagocytophilum*, and *A. platys* may provide a new approach to investigate the host cell tropism of *Anaplasma* spp.

Tr1, a putative transcription factor, is more highly expressed in tick cells infected with *A. phagocytophilum* than in human leukemic HL-60 cells infected with *A. phagocytophilum*, suggesting that Tr1 may regulate genes involved in the bacterial infection cycle in ticks (44, 64). In contrast, Tr is expressed similarly in bovine red blood cells and IDE8 tick cell cultures infected with *A. marginale* (5). Whether the expression of *A. platys* Tr1 differs in infected platelets and tick cells is unknown. In *A. phagocytophilum* tr1, two omp-1s and p44ES are coexpressed (39). In cattle blood, *A. marginale* tr, omp-1, opag1-3, and msp2 are coexpressed (48). OMP-1 homologous proteins are major surface antigens in *Ehrlichia* species (24, 46, 57, 62, 66), and OMP-1X may function similarly in the *A. platys* infection cycle. *A. platys* OMP-1X is predicted to have a β-barrel structure similar to those of *E. chaffeensis* P28 and OMP-1F (37) and is thus probably a porin.

In *A. phagocytophilum* and *A. marginale*, the P44/Msp2 transcript profiles in mammals and ticks are distinct, which may reflect an adaptation to physiological differences between these species (44, 53, 71). Furthermore, conversion of the *A. phagocytophilum* p44 gene within mammalian hosts suggests that P44 plays a role in antigenic variation (8, 20, 38, 65). In cattle, *A. marginale* Msp2 proteins provide the antigenic variation necessary for persistent infection (6, 11, 47). *A. platys* P44 is, therefore, expected to play an important role in determining
persistent or cyclical rickettsemia. It is not known whether *A. platys* p44ES undergoes nonsegmental gene conversion (as in *A. phagocytophilum*) to generate identical P44s from a large number of donor loci) or segmental gene conversion (as in *A. marginale*) to generate mosaicMsp2ES from a small number of donor loci) (39, 47). P44 has a role in the interaction between *A. phagocytophilum* and host cells (36, 49, 64). P44 also has porin activity that allows for passive diffusion of hydrophilic solutes (30). *A. platys* P44 is predicted to have a β-barrel structure similar to that of *A. phagocytophilum* P44 and is thus probably a porin.

*A. phagocytophilum* is known to infect dogs in regions where the *Ixodes* tick is endemic (2, 25, 50, 51). *A. platys* inclusions in the platelets of a naturally infected dog cross-reacted with mouse anti-*A. phagocytophilum* serum (32). It is important, therefore, to develop a method for distinguishing *A. platys* infection from *A. phagocytophilum* infection since the p44 primer pair HVF and HVR, designed in the present study, is specific to *A. platys*, it is expected to be useful for species-specific PCR diagnosis. P44 of *A. phagocytophilum* is the major surface antigen used for serological diagnosis of human granulocytic anaplasmosis (1, 16, 27, 31, 70). In the present study, we could identify several *A. platys*-specific amino acid sequences within P44 proteins that can be used as serologic test antigens to provide differential diagnosis from other *Anaplasma* species infections. Additionally, *Ehrlichia OMP-1/P28/P30/MAP* families are immunodominant major outer membrane proteins that are useful for serodiagnosis (45, 62, 63, 68). Our alignment studies are immunodominant major outer membrane proteins.

We employed touchdown PCR in the present study because the only available source of *A. platys* DNA was a small amount of DNA purified from the blood of naturally infected dogs. Incorrect base calls during the amplification were minimized by using high-fidelity *Taq* polymerase. Only a few *A. platys* gene sequences have previously been reported, including the 16S rRNA, groEL, and gltA genes (3, 33, 67). Application of the molecular approach used here should facilitate the identification of additional DNA sequences to further our understanding of the *A. platys* genome.

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