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NOTE

The rickettsial outer-membrane protein A and B genes of *Rickettsia australis*, the most divergent rickettsia of the spotted fever group

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The genes for rickettsial outer-membrane protein A (rOmpA), a distinguishing feature of spotted fever group (SFG) rickettsiae, and rOmpB, a genus-specific protein, were identified and sequenced in *Rickettsia australis*. The amino acid sequences of domains I, III and IV of the *R. australis* rOmpA share close homology with those of rOmpA of other SFG rickettsiae, but the repeat region (domain II) is dramatically different from that of other known SFG rOmpA. *R. australis* rOmpB is more similar to rOmpB of other SFG rickettsiae than to that of typhus group rickettsiae.

Keywords: *Rickettsia australis*, spotted fever group rickettsiae, rOmpA, rOmpB

The genus *Rickettsia* contains 21 named species and the unnamed AB bacterium (Beati & Raoult, 1993; Beati *et al*., 1993, 1997; Fournier *et al*., 1998; Kelly *et al*., 1996; Roux & Raoult, 1995; Roux *et al*., 1997; Stenos *et al*., 1998; Stothard & Fuerst, 1995; Uchida *et al*., 1992; Weiss & Moulder, 1984; Werren *et al*., 1994). Twelve named species are human pathogens. These organisms belong to either the spotted fever group (SFG) or typhus group (TG). The SFG is characterized as having rickettsial outer-membrane protein A (rOmpA) and distinctive antigenic epitopes on its lipopolysaccharide (Anacker *et al*., 1987; Feng *et al*., 1987; Walker *et al*., 1995; Xu & Raoult, 1998). rOmpA contains a domain consisting of 6–15 near identical tandem repeat units (Anderson *et al*., 1990; Crocquet-Valdes *et al*., 1994; Gilmore, 1993; Walker *et al*., 1995). The main apparent explanation for antigenic differences among SFG rickettsiae including intraspecific diversity is the order and number of the repeat units of rOmpA (Crocquet-Valdes *et al*., 1994; Gilmore, 1993). The TG lacks rOmpA and possesses its own distinctive lipopolysaccharide epitopes (Gilmore & Hackstadt, 1991; Walker *et al*., 1997).

A recent phylogenetic study of 15 strains of SFG rickettsiae reported highly conserved DNA sequences for the regions of rOmpA outside the tandem repeat domain (Fournier *et al*., 1998). Complete rOmpA sequences have been reported only for *Rickettsia rickettsii* and *Rickettsia conorii* (Anderson *et al*., 1990; Crocquet-Valdes *et al*., 1994). Previous attempts to detect r*ompA* in *Rickettsia australis* have been unsuccessful (Eremeeva *et al*., 1994; Fournier *et al*., 1998; Gilmore & Hackstadt, 1991).

The major surface protein of all *Rickettsia* species, rickettsial outer-membrane protein B (rOmpB), possesses conformational epitopes that have been used to classify rickettsial species antigenically (Anacker *et al*., 1987; Carl *et al*., 1990; Dasch, 1981; Gilmore, 1990; Gilmore *et al*., 1989; Walker *et al*., 1995; Xu & Raoult, 1998; Yu *et al*., 1990). rOmpB is a protein of 167 kDa, which is processed after translation to the mature 135 kDa S-layer protein (Carl *et al*., 1990; Gilmore *et al*., 1991; Hackstadt *et al*., 1992; Palmer *et al*., 1974). The complete sequence of r*ompB* has been reported only for *Rickettsia prowazekii*, *Rickettsia typhi*, *R. rickettsii* and *Rickettsia japonica* (Carl *et al*., 1990; Gilmore *et al*., 1991; Hackstadt *et al*., 1992; Palmer *et al*., 1974; GenBank accession no. AB003681).

To determine their phylogenetic relationships, we sequenced r*ompA* and r*ompB* of *R. australis* and r*ompB* of *R. conorii*.

DNA was extracted from *R. australis* PHS and *R. conorii* Malish 7, cultivated in Vero cell culture and purified by Renografin density-gradient centrifugation (Hanson *et al*., 1981). Among overlapping primer pairs designed from sequences of the *R. rickettsii* r*ompA* gene, only one pair [F3-4936 (GGTGGTCAGGCTCTGAAGCTAAC) and B21-6324 (TGCAG-

Abbreviations: SFG, spotted fever group; TG, typhus group.

The GenBank accession numbers for the r*ompA* sequence of *Rickettsia australis* PHS, the r*ompB* sequence of *R. australis* PHS and the r*ompB* sequence of *Rickettsia conorii* Malish 7 are AF149108, AF149109 and AF149110, respectively.

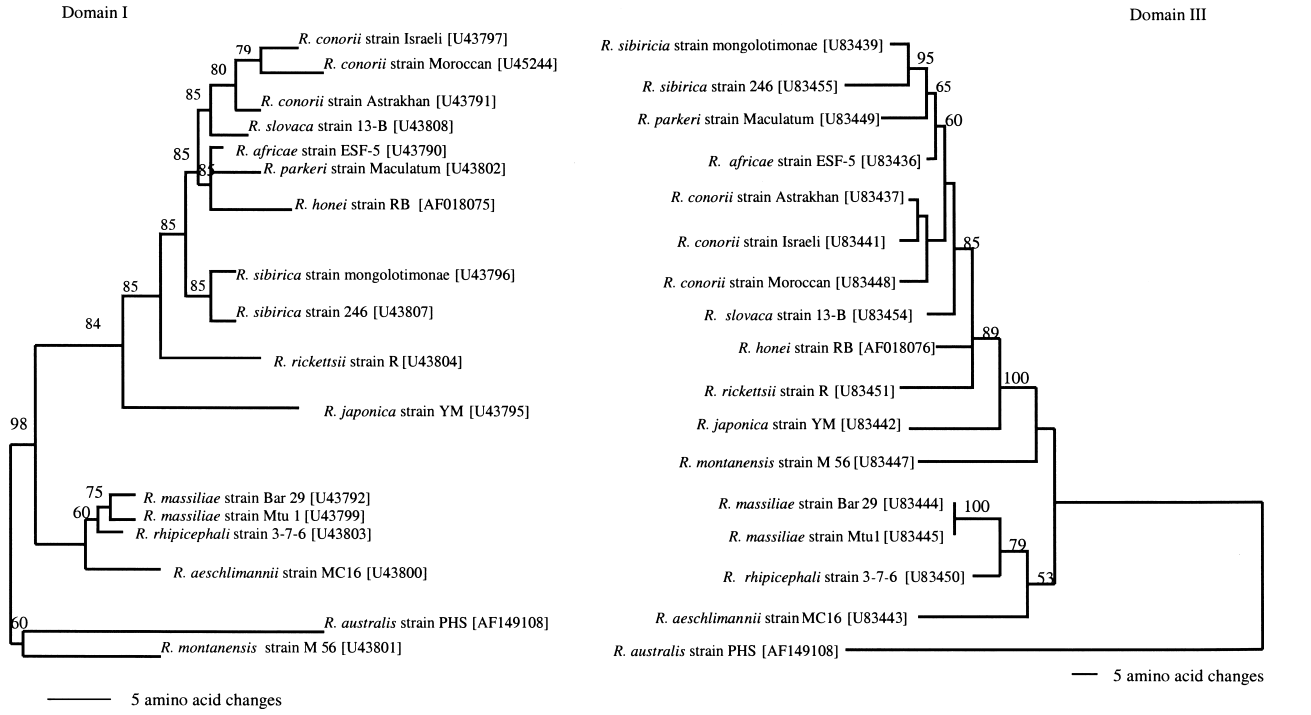

Fig. 1. Phylogenetic tree constructed using sequences of domain I (left) (194 amino acids) and domain II (right) (1000 amino acids) of rOmpA of SFG rickettsial species with bootstrap values that are greater than 50 at the nodes. A total of 17 rickettsial strains were compared. The scale indicates 5 amino acid changes between the species.

TTTGATAACCGACAGTCTC)] yielded a PCR product of 1400 bp with the Advantage genomic PCR kit (Clontech) and 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 68 °C for 1 min. A 3903 bp PCR product of the r*ompB* gene was amplified from *R. australis* and *R. conorii* using primers designed from the *R. rickettsii* r*ompB* gene, RA-120-1S (5′-CGG TCG ACA TGG TTA TAC AAA GTG CTA-3′) and RA-120-2S (5′-CCG TCG ACA TTA GAA GTT TAC ACG GAC-3′). Both upstream and downstream sequences of the *R. australis* r*ompA* gene were determined using a GenomeWalker kit (Clontech), with some modifications.

All PCR products amplified in this study were cloned using the PCR2.1 TA Cloning kit (Invitrogen) and STBL2 competent cells, which contain a unique set of genetic markers allowing for stable cloning of direct repeats. The rickettsial DNA was excised from the PCR2.1 TA vector representing positions 2982–3099 using the flanking *Eco*RI restriction sites and subcloned into pGEM7. The sequence of the repeat region of the *R. australis* r*ompA* was obtained by unidirectional deletion of the insert r*ompA* gene in pGEM7 using the Erase-a-base kit (Promega). All PCR products were sequenced using an ABI PRISM 377 sequencer.

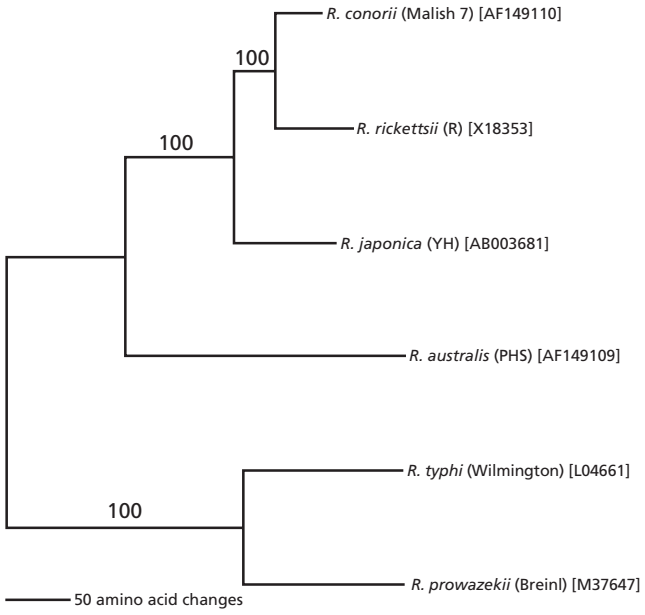

Fig. 2. Phylogenetic tree constructed using amino acid sequences of rOmpB and bootstrap values that are greater than 50 at the nodes. Amino acid sequence derived from a 3·9 kb segment of the *R. australis* template was compared with five other rickettsial species. The scale indicates 50 amino acid changes between the species.

Table 1. Divergence of two rOmpA segments of *R. australis* and other SFG rickettsiae

	R. australis	*R. aeschlimannii*	*R. africae*	*R. conorii*	*R. honei*	*R. japonica*	*R. massiliae*	*R. montanensis*	*R. parkeri*	*R. rhipicephali*	*R. rickettsii*	*R. sibirica*
R. aeschlimannii	0·142857											
R. africae	0·142857	0·048338										
R. conorii	0·148936	0·05279	0·010574									
R. honei	0·147416	0·048265	0·015106	0·019608								
R. japonica	0·147416	0·051282	0·031722	0·036199	0·031674							
R. massiliae	0·139818	0·034691	0·039275	0·043741	0·040724	0·045249						
R. montanensis	0·144377	0·048338	0·039275	0·043807	0·043807	0·048338	0·031722					
R. parkeri	0·153495	0·057315	0·012085	0·019608	0·024133	0·037707	0·045249	0·045317				
R. rhipicephali	0·138298	0·031674	0·036254	0·040724	0·037707	0·042232	0·015060	0·033233	0·045249			
R. rickettsii	0·151976	0·05287	0·019637	0·02568	0·021148	0·033233	0·045317	0·045317	0·027190	0·039275		
R. sibirica	0·162614	0·063348	0·019637	0·027149	0·031674	0·048265	0·054299	0·055891	0·025641	0·051282	0·036254	
R. slovaca	0·144377	0·048265	0·009063	0·013575	0·015083	0·031674	0·039216	0·039275	0·016591	0·036199	0·021148	0·025641

The deduced amino acid sequences were aligned using the MEGALIGN computer program (DNA Star, Madison, WI, USA) based upon residue homology. Phylogenetic analyses were performed using the maximum-parsimony program of the PAUP 4.0 software (Sinauer Associates, Sunderland, MA, USA). Distance matrix analyses were generated using the Kimura two-correction for multiple substitutions. Bootstrap values (Felsenstein, 1985) for the consensus tree were based on analysis of 1000 replicates.

The *R. australis* r*ompA* has a 6318 bp ORF with a repeat region of more than 2 kb composed of eight (255 bp) complete and one (63 bp) incomplete repeat elements. Following the ORF, there is an inverted repeat with identical sequence to that in the homologous region of *R. rickettsii* r*ompA*, occurring 161 bp downstream of the termination codon (TAA) as compared with 21 bp downstream of the *R. rickettsii* r*ompA* termination codon.

The *R. australis* repeat unit differs greatly from the other characterized SFG rickettsial repeat units (Anderson *et al*., 1990), with 21 % identity and 39 % similarity with the amino acid sequence of the *R. rickettsii* type 1 repeat unit. The *R. australis* repeat units seem to be highly conserved among themselves with substitutions occurring in only 9 of the possible 255 nucleotide positions. Of these nucleotide substitutions, only 3 led to amino acid changes. At amino acid 17, alanine was changed to valine in repeats 5, 6 and 8. At amino acid 68, asparagine was changed to threonine in repeats 3, 5 and 8. At amino acid 79, alanine was changed to valine in repeats 3, 5 and 8. These data suggest a fundamental biological function for this region.

GenBank accession numbers for the genes characterized in this study are as follows: *R. australis* (strain PHS) r*ompA*, AF149108; *R. australis* (strain PHS) r*ompB*, AF149109; and *R. conorii* (Malish 7 strain) r*ompB*, AF149110. The GenBank accession numbers of all other rickettsial r*ompA* sequences compared are *R. aeschlimannii* (strain MC16), U43800 and U83443; *R. africae* (strain ESF-5), U43790 and U83436; *R. conorii* (Moroccan strain), U45244 and U83448; *R. conorii* (Astrakhan strain), U43791 and U83437; *R. conorii* (Israeli strain), U43797 and U83441; *R. honei* (strain RB), AF018075 and AF018076; *R. japonica* (strain YM), U43795 and U83442; *R. massiliae* (strain Mtu1), U43799 and U83445; *R. massiliae* (strain Bar 29), U43792 and U83444; *R. montanensis* (strain M56), U43801 and U83447; *R. parkeri* (strain Maculatum), U43802 and U83449; *R. rhipicephali* (strain 3-7-6), U43803 and U83450; *R. rickettsii* (R strain), U43804 and U83451; *R. sibirica* (strain 246), U43807 and U83455; *R. sibirica* (strain mongolotimonae), U43796 and U83439; and *R. slovaca* (strain 13-B), U43808 and U83454. GenBank accession numbers for r*ompB* are *R. conorii* (Malish 7 strain), AF149110; *R. japonica* (YH strain), AB003681; *R. prowazekii* (Breinl strain), M37647; *R. rickettsii* (R strain), X18353; and *R. typhi* (Wilmington strain), L04661.

Phylogenetic trees were constructed using the SFG rickettsial rOmpA amino acid sequence derived from a 584 bp fragment upstream of the repeat region from

Table 2. Divergence of *R. australis* r*ompB* from other Rickettsia species

	R. australis	*R. japonica*	*R. conorii*	*R. rickettsii*	*R. typhi*
R. japonica	0·18048				
R. conorii	0·18070	0·07005			
R. rickettsii	0·19397	0·07935	0·04839		
R. typhi	0·27216	0·27605	0·27393	0·27414	
R. prowazekii	0·27860	0·27393	0·26947	0·27436	0·11309

position 21 to 605 (domain I) and from a 3 kb fragment downstream of the repeat region from position 3290 to 6290 (domain III) (Fig. 1). The phylogenetic trees from two sequences of rOmpA supported each other except for the anomalous placement of *R. montanensis* by domain III sequence analysis. The amino acids encoded by a 3·9 kb segment from both the *R. australis* and *R. conorii* r*ompB* genes were also compared with other rOmpB sequences (Fig. 2).

Tables 1 and 2 summarize the percentage divergence of rOmpA and rOmpB of *R. australis* from the other rickettsial species for which data are available. The rOmpA comparisons indicate that *R. australis* is the most distinct member of the SFG with percentage divergences ranging from 13·8 to 16·3%. The rOmpB comparisons display larger percentage divergences (18·1–19·4%) of *R. australis* from the other SFG rickettsiae, and greater divergence (27·2–27·9%) when compared with the TG rickettsiae. These results have defined the extreme limit of the SFG among the strains of rickettsiae evaluated at present.

The taxonomic implications of these sequence data for r*ompA* (only the third reported complete sequence) and r*ompB* (the fifth and sixth determined sequences) are significant. The existence of r*ompA* in *R. australis* confirms the conclusion from its lipopolysaccharide epitopes shared with other SFG rickettsiae, namely that *R. australis* is a member of the SFG. The close relatedness of *R. conorii* and *R. rickettsii*, only 4·8% divergence of r*ompB* as compared with 11·3% divergence between *R. typhi* and *R. prowazekii*, would suggest that careful consideration should be given to whether they and the other rickettsial strains and named species in their clade, including *R. sibirica*, *R. slovaca*, *R. parkeri*, *R. africae*, and the Israeli and Astrakhan strains, might represent strains of a single species. The differences in the placements of the organisms in this clade between the analyses of domains I and III of rOmpA also suggest that there may be more taxonomic names than actual *Rickettsia* species. Similar concepts are suggested by phylogenetic relationships demonstrated by the citrate synthase, 17 kDa protein and 16S rRNA genes (Billings *et al.*, 1998; Roux & Raoult, 1995; Roux *et al.*, 1997; Stothard & Fuerst, 1995).

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