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Restriction site polymorphisms in the genes encoding new members of group 3 outer membrane protein family of *Brucella* spp.

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Abstract

Thirty-seven *Brucella* reference and field strains representing all the species and their biovars were analysed by PCR–RFLP to determine the degree of variation in the genes encoding the new members of group 3 outer membrane protein (Omp) family. Analysis of the *omp22* and *omp25c/omp25d* genes indicated that the restriction patterns were identical for all species and biovars with all restriction enzymes tested, except for *Brucella ovis* that showed a short 30 bp deletion close to *omp22* gene, and for *B. abortus* biovar 6 and *B. ovis* that lacked a *Dde*I site and a *Hinf*I site, respectively, in the *omp25c/omp25d* genes. Analysis of PCR products of the *omp31b* gene digested with 20 restriction enzymes revealed that this gene has a greater level of DNA polymorphism than the other genes encoding the new members of group 3 Omp family. A deletion of 232 bp was detected in fourteen *B melitensis* strains from different hosts and from different geographic origins, confirming that this feature is indeed a hallmark of *B. melitensis*. PCR–RFLP analysis of *omp31b* with *Dde*I allowed us to identify species-specific markers for *B. abortus*, *B. melitensis*, and *B. ovis*. Finally, by PCR analysis, Southern blot hybridization and DNA sequencing we showed that a large deletion of 15 kb, comprising the entire *omp25b* gene and 21 more genes, is present in all *B. ovis* strains, thus confirming previous observations from other authors.

Keywords: *Brucella*; DNA polymorphism; Outer membrane proteins

1. Introduction

The genus *Brucella* consists of six species, designated on the basis of differences in pathogenicity and host preference as *Brucella melitensis* (goats and sheep), *B. abortus* (cattle and bison), *B. suis* (infecting primarily swine, but also hares, rodents and reindeer), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (wood rats) [1]. The discovery of *Brucella* bacteria in marine mammals has led to the proposal of two additional species: *B. cetaceae*, infecting cetaceans, and *B. pinnipediae*, infecting pinnipeds [2]. By an analysis of approximately 25 phenotypic characteristics, including serological typing for lipopolysaccharide, phage typing, sensitivity to dyes, requirement for CO_2, H_2S production and metabolic properties, the genus *Brucella* can be classified in species and biovars [3]. However, some problems are associated with these tests. Thus, it takes more than one week to culture the bacteria and complete the typing, the tests require skilled technicians, and handling of the microorganism represents a high risk for laboratory personnel since most *Brucella* strains are highly pathogenic [4]. In an attempt to overcome these difficulties, several techniques have been employed in order to identify DNA polymorphisms that would enable

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molecular typing of *Brucella* (reviewed by Vizcaíno et al. [5]). In this regard, studies of the restriction fragment length polymorphisms (RFLP) of genes coding for the outer membrane proteins Omp2a and Omp2b (known as group 2 Omps with 36–38 kDa), and Omp25 and Omp31 (known as group 3 Omps with 25–31 kDa) have allowed the differentiation between the *Brucella* species and biovars [2,6–8]. Specifically, PCR–RFLP analysis of *omp25* revealed that *B. melitensis* strains lack a conserved *Eco* RV site and *B. ovis* strains have a 36 bp deletion at the 3′ end of the gene [6]. In addition, a large DNA deletion leading to the loss of the entire *omp31* gene has been shown to be present in all *B. abortus* strains, and PCR–RFLP of *omp31* revealed specific markers for *B. canis*, *B. ovis* and *B. suis* biovar 2 [8].

Analysis of the completed *B. melitensis* and *B. suis* genomes revealed the presence of five new genes homologous to *omp25* and *omp31*, and indicated the existence of new members of the group 3 Omp family [9]. Salhi et al. [10] have classified these seven Omps in four subgroups based on their homology at the amino acid level: Omp25, Omp22, the Omp25b-Omp25c-Omp25d cluster, and the Omp31-Omp31b subgroup. It has been shown that all these new members of group 3 Omps are expressed in *B. suis*, *B. abortus* or *B. melitensis* [9–11]. In the present study, we have determined, by PCR–RFLP, the existence of DNA polymorphisms in the *omp22*, *omp25b*, *omp25c*, *omp25d* and *omp31b* genes of 37 *Brucella* reference and field strains, including representative strains of all the species and its biovars.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The *Brucella* strains used in this study are listed in Table 1. Cultures were grown on Trypticase Soy Agar

Table 1
Brucella strains used in this study

Species	Biovar	Strain[a]	Host/origin
B. abortus	1	2308	Bovine/USA
	1	RB51	–/USA
	1	45/20	Bovine/USA
	2	86/8/59 (ATCC 23449)	Bovine/UK
	3	Tulya (ATCC 23450)	Human/Uganda
	4	89.57	Bovine/France
	5	B3196 (ATCC 23452)	Bovine/UK
	6	88.21	Bovine/France
	9	C68 (ATCC 23455)	Bovine/UK
B. melitensis	1	16M (ATCC 23456)	Goat/USA
	1	H38	Goat/Mexico
	1	115	–
	1	Rev1	Goat/USA
	1	1507	Goat/Spain
	1	1534	Human/Spain
	2	63/9 (ATCC 23457)	Goat/India
	2	1449	Goat/Spain
	2	1461	Goat/Spain
	2	1455	Human/Spain
	3	Ether (ATCC 23458)	Human/Italy
	3	1549	Human/Spain
	3	1553	Bovine/Spain
	3	1559	Goat/Spain
B. suis	1	78.145	Not known
	2	Thomsen1 (ATCC 23445)	Porcine/Denmark
	3	686 (ATCC 23446)	Human/USA
	4	87.59	not known
	5	ELT80	Human/USA
B. ovis		63/290 Bow (ATCC 25840)	Ovine/Australia
		Reo198	Ovine/USA
		PA	Ovine/France
		2306	Ovine/Spain
		2206	Ovine/France
B. canis		RM6/66 (ATCC 23365)	Dog/USA
B. neotomae		5K33 (ATCC 23459)	Desert wood rat/USA
B. pinnipediae		B2/94	Seal/Scotland
B. cetaceae		B14/94	Dolphin/Scotland

[a] ATCC: American type culture collection.