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Rapid Identification of Rough *Brucella* Isolates by a Latex Coagglutination Assay with the 25-Kilodalton Outer Membrane Protein and Rough-Lipopolysaccharide-Specific Monoclonal Antibodies

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A latex coagglutination assay was developed to identify rough (R) isolates of *Brucella*. Latex beads were coated, via protein A, with either an anti-*Brucella* rough-lipopolysaccharide (R-LPS) monoclonal antibody (MAb) or an anti-*Brucella* 25-kDa outer membrane protein (Omp25) MAb. Slide agglutination tests were done for 68 strains of *Brucella* spp., including type strains of all biovars as well as field isolates. Latex beads coated with MAb to R-LPS coagglutinated only R strains, whereas latex beads coated with MAb to Omp25 coagglutinated all the R *Brucella* isolates except *Brucella ovis*. Coagglutination was easier to read than agglutination with rabbit R-*Brucella*-specific antiserum. Thus, this assay accurately differentiates *B. ovis* from other R *Brucella* isolates. The latex coagglutination assay can substitute, to advantage, for the current anti-*Brucella* (R) rabbit monospecific serum.

The method for identification of *Brucella* isolates includes steps intended to determine genus, species, and, finally, biovar. One of these steps is identification of the phase of the culture. This is determined by physical methods as well as by slide agglutination with monospecific antisera (2). However, preparation of stocks of rabbit monospecific antisera is laborious and time-consuming, requiring adsorption steps with *Brucella* suspensions and a careful titration with smooth (S) and rough (R) *Brucella* strains. In addition, sometimes agglutination is weak, requiring further testing. Monoclonal antibodies (MAbs) to *Brucella* have been developed and used to characterize surface molecules, including smooth lipopolysaccharide (S-LPS) and rough lipopolysaccharide (R-LPS) and major and minor outer membrane proteins (OMP) (6, 8, 9). It has been shown that MAbs to both R-LPS and OMP bind better to R than to S *Brucella* cells (6, 8). In addition, some surface-exposed epitopes, present in the conserved, major OMP, Omp25 (10, 11), are lacking in the Omp25 from *Brucella ovis* (10). On the basis of these data, we developed a coagglutination test which can substitute for monospecific rabbit antisera in the determination of colony properties and which differentiates *B. ovis* from other ovine and nonovine R *Brucella* isolates.

The bacteria tested in this work and their sources, hosts, and geographical origins are listed in Table 1 (*Brucella*) and 2 (non-*Brucella*). *Brucella* strains were grown for 24 h in tryptic soy agar (Gibco BRL) supplemented with yeast extract (Difco) (TSAYE); for *B. ovis* and *B. abortus* biovar 2, TSAYE was enriched with 5% horse serum (Gibco, Scotland), and 10% CO_2 was provided for some species as recommended (2). The other genera were grown in TSAYE. MAbs were A68/03F03/D05 (immunoglobulin G2b) (8, 9) and A76/02C12/C11 (immunoglobulin G2a) (10, 11), which are specific for *Brucella* R-LPS and *Brucella* Omp25, respectively. The preparation of latex suspensions was as follows. A 50-μl volume of a 10% suspension of latex calibrated particles (Estapor, 0.8 μm; Rhône-Poulenc Ltd., Manchester, United Kingdom) (4) was washed twice with a 0.5-ml volume of 20 mM glycine–34 mM NaCl–6.15 mM NaN_3 and incubated in the same buffer with 100 μg of protein A (catalog no. P-6031; Sigma, St. Louis, Mo.) for 1 h at room temperature (22°C) in a total volume of 0.5 ml. Latex was washed once with 1 ml of 100 mM glycine–170 mM NaCl–6.15 mM NaN_3 (GBS) and incubated with 1.5 ml of hybridoma supernatant (antibody concentration, 50 μg/ml) for 1 h at room temperature. Finally, latex was washed three times with 1 ml of 1% bovine serum albumin in GBS, resuspended in 1 ml of this buffer, and stored frozen (−20°C). For testing latex coagglutination, fresh bacterial cultures were used. A loopful of a selected colony and a 20-μl drop of latex suspension (which was previously vigorously vortexed) were placed close together onto a glass slide. Bacteria were mixed with the latex with glass rods, and incubation proceeded for 2 min. Agglutination was determined by direct visual examination while the slide was gently rocked and expressed qualitatively as "+" (agglutination) or "−" (no agglutination). For comparison, each strain was tested in parallel with an anti-*Brucella* (R) rabbit monospecific serum (2). When necessary, weak agglutinations were confirmed by examination through a binocular stereomicroscope.

Latex coagglutination tests were conducted for 68 *Brucella* sp. strains (18 reference and 50 field strains from different hosts and geographical origins). Specificity was assessed by testing 15 non-*Brucella* strains, including bacteria reported to be related to *Brucella* antigenically (*Yersinia enterocolitica* O:9, *Salmonella urbana*, *Escherichia coli* O:157, and *Francisella tularensis*) (2) or genetically (*Ochrobactrum anthropi* and *Phyllobacterium* spp.) (12). Two latex preparations were used, one prepared with *Brucella* R-LPS-specific MAb A68/03F03/D05 (LxR) and the other prepared with *Brucella* Omp25-specific MAb A76/02C12/C11 (Lx25).

None of the S *Brucella* strains coagglutinated with either LxR or Lx25. All of the 44 R *Brucella* strains coagglutinated

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TABLE 1. Results for S and R *Brucella* strains tested in a coagglutination assay with latex beads coated with R-LPS-specific (LxR) and Omp25-specific (Lx25) MAbs

Species and biovar	Strain	Colony phase	Source[a]	Host or derivation	Geographic origin	Agglutination with latex prepn[b]:	
						LxR	Lx25
Reference strains							
B. melitensis bv. 1	16M	S	ATCC	Goat	United States	−	−
B. melitensis bv. 2	63/9	S	ATCC	Goat	Turkey	−	−
B. melitensis bv. 3	Ether	S	ATCC	Goat	Italy	−	−
B. abortus bv. 1	544	S	ATCC	Cattle	England	−	−
B. abortus bv. 2	86/8/59	S	ATCC	Cattle	England	−	−
B. abortus bv. 3	Tulya	S	ATCC	Human	Uganda	−	−
B. abortus bv. 4	292	S	ATCC	Cattle	England	−	−
B. abortus bv. 5	B3196	S	ATCC	Cattle	England	−	−
B. abortus bv. 6	870	S	ATCC	Cattle	Africa	−	−
B. abortus bv. 9	C68	S	ATCC	Cattle	England	−	−
B. suis bv. 1	1330	S	ATCC	Swine	United States	−	−
B. suis bv. 2	Thomsen	S	ATCC	Swine	Denmark	−	−
B. suis bv. 3	686	S	ATCC	Swine	United States	−	−
B. suis bv. 4	40	S	ATCC	Reindeer	Former USSR	−	−
B. suis bv. 5	513	S	BCCN	Wild rodent	Former USSR	−	−
B. neotomae	5K33	S	ATCC	Desert rat	United States	−	−
B. ovis	63/290	R	ATCC	Sheep	Africa	+	−
B. canis	RM6/66	R	ATCC	Dog	United States	+	+
Field strains							
B. melitensis bv. 1	87-92 (EP)	S-R	BCCN	Human	United States	+	+
B. melitensis	H38R	R	BCCN	Variant from H38		+	+
	Rev.1R	R	BCCN	Variant from Rev.1		+	+
	B115	R	BCCN	Goat	Malta	+	+
	82-61	R	BCCN	Human	Spain	+	+
	82-71	R	BCCN	Human	Spain	+	+
	83-21	R	BCCN	Human	France	+	+
	91-269	R	BCCN	Goat	France	+	+
	92-85	R	BCCN	Sheep	Spain	+	+
	92-119	R	BCCN	Human	Tunisia	+	+
	93-40	R	BCCN	Human	Palestine	+	+
B. abortus	544R	R	BCCN	Variant from 544		+	+
	S19R	R	BCCN	Variant from S19		+	+
	45/20	R	BCCN	Cattle	England	+	+
	RB51	R	BCCN	Variant from 2308		+	+
	75-468	R	BCCN	Cattle	France	+	+
	88-35	R	BCCN	Horse	Tunisia	+	+
	76-403	R	BCCN	Human	France	+	+
B. suis	174	R	INM	Human	Argentina	+	+
	300B	R	INM	Human	Argentina	+	+
B. ovis	Reo 198	R	BCCN	Sheep	United States	+	−
	74-306	R	BCCN	Sheep	France	+	−
	74-307	R	BCCN	Sheep	France	+	−
	74-315	R	BCCN	Sheep	France	+	−
	74-323	R	BCCN	Sheep	France	+	−
	74-326	R	BCCN	Sheep	France	+	−
	74-331	R	BCCN	Sheep	France	+	−
	74-335	R	BCCN	Sheep	France	+	−
	74-341	R	BCCN	Sheep	France	+	−
	74-346	R	BCCN	Sheep	France	+	−
	76-247	R	BCCN	Sheep	France	+	−
	76-250	R	BCCN	Sheep	France	+	−
	78-256	R	BCCN	Sheep	France	+	−
	81-143	R	BCCN	Sheep	Spain	+	−
	91-65	R	BCCN	Sheep	Spain	+	−
	91-69	R	BCCN	Sheep	Spain	+	−
	91-206	R	BCCN	Sheep	Spain	+	−
	91-211	R	BCCN	Sheep	Spain	+	−
	91-264	R	BCCN	Sheep	Argentina	+	−
	91-265	R	BCCN	Sheep	Argentina	+	−
	91-266	R	BCCN	Sheep	Argentina	+	−
	91-267	R	BCCN	Sheep	Argentina	+	−

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TABLE 1—*Continued*

Species and biovar	Strain	Colony phase	Source[a]	Host or derivation	Geographic origin	Agglutination with latex prepn[b]:	
						LxR	Lx25
B. canis	D519	R	BCCN	Dog	Madagascar	+	+
	H966	R	BCCN	Dog	United States	+	+
	Hoy 1066	R	BCCN	Dog	United States	+	+
	315	R	BCCN	Dog	United States	+	+
	87-62	R	BCCN	Dog	Canada	+	+
	87-66	R	BCCN	Dog	Canada	+	+
Brucella spp.	94-73	S	BCCN	Seal	Scotland	−	−
	94-74	S	BCCN	Porpoise	Scotland	−	−
	94-75	S	BCCN	Dolphin	Scotland	−	−

[a] ATCC, American Type Culture Collection; BCCN, Brucella Culture Collection Nouzilly, Nouzilly, France; INM, Instituto Nacional de Microbiologia, Buenos Aires, Argentina.
[b] −, no agglutination; +, agglutination.

with LxR. Although Lx25 coagglutinated R *Brucella* well, this preparation failed consistently to do so with all the *B. ovis* strains tested ($n = 22$). S-R *B. melitensis* EP (5) behaved in this assay like R *B. melitensis*, although clumping with Lx25 was weaker. This was most likely due to steric hindrance provoked by S-LPS chains present in small amounts in this strain (5).

Neither LxR nor Lx25 coagglutinated the non-*Brucella* strains tested. Monospecific anti-*Brucella* (R) serum agglutinated only R and S-R *Brucella* strains. Coagglutinations with both LxR and Lx25 were clear and easier to read than agglutination with monospecific serum; however, LxR gave a more intense and rapid reaction than Lx25. Only with monospecific serum were agglutinations further observed with a stereomicroscope.

Brucellosis in sheep can be due to infection by *B. melitensis* or *B. ovis* (2, 3). Although *B. melitensis* is an S species, R forms can sometimes be isolated from infected sheep (1, 3). Rapid identification of *Brucella* includes a study of colony morphology, including the surface, and reactivity of fresh suspensions made from the colony with specific antisera. Although agglutination with anti-*Brucella* S (A or M O-polysaccharide chain) antisera could be one criterion for differentiating S *B. melitensis* from *B. ovis*, this cannot distinguish between the latter and R forms of *B. melitensis* or even of other species. Indeed, agglutination tests with anti-*Brucella* (R) serum can only confirm the colony as R *Brucella*.

The method presented in this work relies on the use of a pair of MAbs, directed against the R-LPS and Omp25, respectively, of *Brucella*. The epitope recognized by MAb A76/02C11/C12 (Lx25) is present in all *Brucella* species except *B. ovis* and is surface exposed (10). In *B. ovis*, a different conformation of the protein due to a deletion in the *omp25* gene explains the absence of the A76/02C11/C12 epitope (10). Other authors previously had reported the usefulness of coagglutination with monospecific (A or M) anti-S-LPS antisera (13) or anti-S-LPS (A) (14) or (M) (15) MAbs in typing S *Brucella* cultures. However, non-S cultures could not be positively typed with those reagents.

In conclusion, using latex beads coated with this pair of MAbs, we were able to distinguish *B. ovis* isolates from other S and R *Brucella* isolates. The test was determined to be specific for the genus *Brucella* as no reactivity was observed with other genetically or antigenically related organisms. In addition, LxR can be used alone as a substitute for anti-R monospecific serum, giving clear-cut results with the advantages of MAb-based technology (7).

TABLE 2. Non-*Brucella* organisms used in this study[a]

Bacterium and strain[b]
Yersinia enterocolitica O:9 Ye8
Escherichia coli O:111 Ec1
Escherichia coli O:157 Ec2
Salmonella urbana Su1
Campylobacter fetus subsp. *fetus*
Campylobacter fetus subsp. *venerealis*
Francisella tularensis Ft1
Ochrobactrum anthropi Oa1
Ochrobactrum anthropi Oa2
Ochrobactrum anthropi Oa3
Alcaligenes denitrificans Ad1
Xanthomonas maltophilia Xm1
Agrobacterium radiobacter Ar1
Agrobacterium tumefaciens At1
Phyllobacterium rubiacearum Pr1
Phyllobacterium myrcinacearum Pm1
Rhizobium leguminosarum R11

[a] Bacteria were tested with latex beads coated with R-LPS-specific (LxR) and Omp25-specific (Lx25) MAbs to assess the specificity of the coagglutination assay. There was no agglutination for any of the non-*Brucella* organisms tested.
[b] Strain denominations are those given at INRA, Nouzilly, France. Many of these strains were originally obtained from Laboratoire Central de Recherches Vétérinaires, Centre National d'Etudes Vétérinaires et Alimentaires, Maisons-Alfort, France; Facultad de Ciencias Veterinarias, UNICEN, Tandil, and Fundación Investigación Biológica Argentina, Mar del Plata, Argentina; and Institut Pasteur, Paris, and Laboratoire de Touraine, Tours, France.

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