Brucella inopinata sp. nov., isolated from a breast implant infection

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A Gram-negative, non-motile, non-spore-forming coccoid bacterium (strain BO1^T) was isolated recently from a breast implant infection of a 71-year-old female patient with clinical signs of brucellosis. Affiliation of strain BO1^T to the genus *Brucella* was confirmed by means of polyamine pattern, polar lipid profile, fatty acid profile, quinone system, DNA–DNA hybridization studies and by insertion sequence 711 (IS711)-specific PCR. Strain BO1^T harboured four to five copies of the *Brucella*-specific insertion element IS711, displaying a unique banding pattern, and exhibited a unique 16S rRNA gene sequence and also grouped separately in multilocus sequence typing analysis. Strain BO1^T reacted with *Brucella* M-monospecific antiserum. Incomplete lysis was detected with bacteriophages Tb (Tbilisi), F1 and F25. Biochemical profiling revealed a high degree of enzymic activity and metabolic capabilities. In multilocus VNTR (variable-number tandem-repeat) analysis, strain BO1^T showed a very distinctive profile and clustered with the other 'exotic' *Brucella* strains, including strains isolated from marine mammals, and *Brucella microti*, *Brucella suis* biovar 5 and *Brucella neotomae*. Comparative *omp2a* and *omp2b* gene sequence analysis revealed the most divergent *omp2* sequences identified to date for a *Brucella* strain. The *recA* gene sequence of strain BO1^T differed in seven nucleotides from the *Brucella recA* consensus sequence. Using the *Brucella* species-specific multiplex PCR assay, strain BO1^T displayed a unique banding pattern not observed in other *Brucella* species. From the phenotypic and molecular analysis it became evident that strain BO1^T was clearly different from all other *Brucella* species, and therefore represents a novel species within the genus *Brucella*. Because of its unexpected isolation, the name *Brucella inopinata* with the type strain BO1^T (=BCCN 09-01^T=CPAM 6436^T) is proposed.

The genus *Brucella* was constituted in 1920 by Meyer & Shaw (1920) with *Brucella melitensis*, the causative agent of Malta fever, as the type species and *Brucella abortus* causing abortion in cattle as the second species. Within 48 years four additional *Brucella* species, namely *Brucella suis* (Huddleson, 1929), *Brucella ovis* (Buddle, 1956), *Brucella neotomae* (Stoenner & Lackman, 1957) and *Brucella canis* (Carmichael & Bruner, 1968) were described. It took nearly another 40 years before two additional species, *Brucella pinnipedialis* and *Brucella ceti*, originating from marine mammals were published (Foster *et al.*, 2007). The most recently published description of a *Brucella* species is that for *Brucella microti*, isolated initially from common voles in the Czech Republic (Hubalek *et al.*, 2007; Scholz *et al.*, 2008a) and recently also from red foxes in Lower Austria (*Vulpes vulpes*) and directly from soil (Scholz *et al.*, 2009, 2008c).

Before the development of molecular techniques, differentiation of the various *Brucella* species and their biovars was solely based on phenotypic traits, i.e. CO₂ requirement, H₂S production, dye sensitivity, metabolic profiles, lysis by *Brucella*-specific bacteriophages and agglutination with monospecific antisera. Another important criterion for the differentiation of the various *Brucella* species was the natural host preference, which is why species names were given in accordance with their preferred host. Today it is known that all *Brucella* species are highly related at the genetic level, exhibiting identical 16S rRNA (Gee *et al.*, 2004) and *recA* gene sequences (Scholz *et al.*, 2008b) as well as highly similar genomes in terms of sequence identity and gene synteny (Del Vecchio *et al.*, 2002; Paulsen *et al.*, 2002; Halling *et al.*, 2005). In the majority of housekeeping genes only single nucleotide polymorphisms are present among the various *Brucella* species (Whatmore *et al.*, 2007). Indeed, applying the gold standard for species delineation, DNA–DNA hybridization (Wayne *et al.*, 1987), all recognized *Brucella* species belong to a single species, as the DNA–DNA relatedness among all species is above 70 % (Verger *et al.*, 1985). Consequently, Verger *et al.* (1985) suggested *Brucella* as a monospecific genus with *B. melitensis* as the sole true species and the other species to be recognized as biovars. This perception was later confirmed by using data from comparative whole genome

sequencing analysis of *B. melitensis* (Del Vecchio *et al.*, 2002), *B. suis* (Paulsen *et al.*, 2002), and more recently of *Brucella abortus* strain 9-941 (Halling *et al.*, 2005) and *B. abortus* vaccine strain S19 (Crasta *et al.*, 2008).

However, in 2003, the Subcommittee on the Taxonomy of *Brucella* agreed unanimously on a return to pre-1986 *Brucella* taxonomy and reapproval of the six *Brucella* nomenspecies and their biovars (Meeting in Pamplona, Spain in 2003), published in 2006 by Osterman & Moriyón (2006).

Following the currently used *Brucella* taxonomy we describe a novel *Brucella* species, *Brucella inopinata* sp. nov., with the type strain BO1^T, isolated from breast implant wound fluid and blood of a 71-year-old female patient with clinical symptoms consistent with brucellosis (De *et al.*, 2008).

B. inopinata sp. nov. is the first *Brucella* species that exhibits a unique 16S rRNA gene sequence and lower sequence similarities in various housekeeping genes and genes encoding outer-membrane proteins when compared with all other recognized *Brucella* species and hence represents the most unique species within this genus.

Phenotypic analysis of strain BO1^T

Phenotypic analysis of strain BO1^T, i.e. growth behaviour on different media at various temperatures, H₂S production, CO₂ requirement, agglutination with monospecific A- and M-antisera, cellular fatty acid analysis, lysis with bacteriophage Tb and growth in the presence of dyes (thionin and basic fuchsin), have been described previously (De *et al.*, 2008) and are given in the species description. The antimicrobial susceptibility pattern of strain BO1^T was determined using the CLSI interpretive criteria for *Brucella* spp. (De *et al.*, 2008). Results are given in the species description. Additional phenotypic characterization of strain BO1^T carried out in this study comprised other growth characteristics, standard biochemical characterization by using API 20E, API 20NE and API ZYM (bioMérieux), extended biochemical profiling using the MICRONAUT (MERLIN Diagnostika GmbH) system, electron microscopy, and lysis with additional *Brucella*-specific bacteriophages, F1 and F25, as described for *B. microti* (Scholz *et al.*, 2008a).

The growth characteristics of strain BO1^T resembled those of *B. microti*, characterized by very fast growth at 37 °C on various standard media, such as sheep blood agar and standard nutrient agar (both from Oxoid). On *Brucella* agar (Merck), growth became visible within 6 h of incubation at 37 °C with or without supplementary CO₂ (data not shown). Single colonies of 1–2 mm were formed within 12–24 h. As determined by using transmission electron microscopy (negative staining with 1 % uranyl acetate) with a JEM-1010 electron microscope at a magnification of 40 000, cells were non-flagellated and arranged individually or in irregular clusters, exhibiting a

Abbreviations: MLST, multilocus sequence typing; MLVA, multilocus VNTR (variable-number tandem-repeat) analysis; RTD, routine test dilution.

The GenBank/EMBL/DBJ accession numbers for the *omp2a*, *omp2b*, *omp25*, *omp31* and *recA* gene sequences of strain BO1^T are FM177715, FM177716, FM177717, FM177718 and FM177719, respectively.

Figures showing lysis of strain BO1^T on *Brucella* agar by bacteriophage Tb (Tbilisi), a two-dimensional thin-layer chromatogram of total polar lipids of strain BO1^T, IS711 element fingerprinting by using Southern blotting and a condensed dendrogram of clustered MLVA-16 genotypes obtained with more than 470 *Brucella* isolates corresponding to 324 different genotypes are available as supplementary material with the online version of this paper.

mean diameter of 0.5 μm and a length of 1 μm (Fig. 1a and b). Strain BO1^T agglutinated weakly with monospecific anti-M serum up to a dilution of 1 : 40 but not with anti-A serum. Incomplete lysis was observed with bacteriophages Tb, F1 and F25 at $10^4 \times$ routine test dilution ($10^4 \times$ RTD) but not at any other dilutions (Supplementary Fig. S1, in IJSEM Online). This finding is in contradiction to previous results (De *et al.*, 2008), in which no lysis could be detected with phage Tb. This can be explained by the rapid growth of strain BO1^T. In order to detect lysis, the standard protocol had to be modified in terms of bacteria quantities and incubation periods. Briefly, *Brucella* agar plates were flooded with 5 ml *Brucella* bouillon including 5 μl of a 10^{11} cell suspension of BO1^T ml^{-1} . Medium in excess was removed immediately and the plates were dried at 37 °C for 2 h before the various phage dilutions were applied. The incubation time of the phage dilutions was 10 s. Plates were incubated at 37 °C and inspected for phage lysis after 12 h.

As described previously for *B. microti* (Scholz *et al.*, 2008a), strain BO1^T exhibited outstanding metabolic capabilities in comparison with recognized *Brucella* species, sharing a series of reactions with *Ochrobactrum anthropi* LMG 3331^T and *Ochrobactrum intermedium* LMG 3301^T. The results of the differential biochemical reactions of strain BO1^T in

comparison with other *Brucella* species and their biovars as well as with *Ochrobactrum* species are summarized in Table 1. Hence, *B. inopinata* sp. nov. is the second fast-growing *Brucella* species with a highly active metabolism.

Using the API 20E and API 20NE test systems, strain BO1^T was misidentified as *Ochrobactrum anthropi* with identification scores of 98 and 99 %, respectively. Detailed results of API 20E, API 20NE, and API ZYM test systems are given in the species description.

Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger *et al.* (1996). The quinone system consisted of the major compound ubiquinone Q-10 (99 %) and ubiquinone Q-11 (1 %). The polar lipid profile consisted of five major compounds: phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine and one unknown aminolipid (Supplementary Fig. S2, in IJSEM Online). The unknown aminolipid exhibited the same chromatographic behaviour as the unknown aminolipid AL1, which was also reported to be present in the type strains of *B. microti*, *B. melitensis* and *B. abortus* (Scholz *et al.*, 2008a). In addition, moderate amounts of phosphatidylmonomethylethanolamine and minor to trace amounts of an unknown aminophospholipid and an unknown phospholipid corresponding to APL2 and PL, respectively, which were detected in *B. microti*, as well as four unknown polar lipids were detected. One of these unknown polar lipids showed the same chromatographic behaviour as the lipid L4 present in *B. microti* but not in other *Brucella* species (Scholz *et al.*, 2008a). Unknown aminolipid AL2 found in *B. microti* (Scholz *et al.*, 2008a) as a major compound was not detectable. Hence, the polar lipid profile clearly distinguished strain BO1^T from *B. microti* as well as from *B. melitensis* and *B. abortus* based on the presence/absence of unknown aminolipid AL2 and unknown polar lipid L4. The polyamine pattern contained the major compounds spermidine (45 %) and putrescine (43 %) and small amounts of 1,3-diaminopropane (7 %), *sym*-homospermidine (1 %) and spermine (1 %). This polyamine pattern is in good agreement with those of other *Brucella* species (Scholz *et al.*, 2008a).

The whole-cell cellular fatty acid profile of strain BO1^T was similar to those of *B. abortus*, *B. melitensis*, *B. neotomae*, *B. ovis* and *B. suis*, characterized by the presence of major amounts (5–46 %) of C_{19:0} cyclo 11-12, C_{18:1 ω 7c}, C_{18:0} and C_{16:0} and smaller amounts (1–4 %) of Br-C_{19:1}. The antimicrobial susceptibility pattern of strain BO1^T was comparable with profiles of other *Brucella* species described by Jevitt *et al.* (2005), with the exception of resistance to various cephalosporins (cefoxitin, cephotiam and cefuroxime). Details are given in the species description.

Molecular analyses of strain BO1^T

Previous molecular analyses of strain BO1^T comprised 16S rRNA gene sequencing, DNA–DNA hybridization experi-

Fig. 1. Transmission electron micrographs (magnification of 40 000) showing two inactivated non-flagellated *B. inopinata* BO1^T cells (a) and a cluster of *B. inopinata* BO1^T cells (b). Bars, 0.2 μm (a) and 1 μm (b).

Table 1. Differential physiological reactions of *B. inopinata* sp. nov. BO1^T in comparison with other *Brucella* and *Ochrobactrum* species

Taxa: 1, *B. microti* CCM 4915^T; 2, *B. inopinata* sp. nov. BO1^T; 3, *B. abortus* strains: 544^T (=NCTC 10093^T) (bv. 1), 86/8/59 (=NCTC 10501) (bv. 2), Tulya (=NCTC 10502) (bv. 3), 292 (=NCTC 10503) (bv. 4), B3196 (=NCTC 10504) (bv. 5), 870 (=NCTC 10505) (bv. 6), 63175 (=NCTC 10506) (bv. 7), C68 (=NCTC 10507) (bv. 9); 4, *B. melitensis* strains: 16M^T (=NCTC 10094^T) (bv. 1), 63/9 (=NCTC 10508) (bv. 2), Ether (=NCTC 10509) (bv. 3); 5, *B. suis* strains: 1330^T (=NCTC 10316^T) (bv. 1), Thomsen (=NCTC 10510) (bv. 2), 686 (=NCTC 10511) (bv. 3), reference 40 (bv. 4), reference 513 (bv. 5); 6, *B. ovis* 63/290^T (=NCTC 10512^T); 7, *B. canis* RM6/66^T (=NCTC 10854^T); 8, *B. neotomae* 5K33^T (=NCTC 10084^T); 9, *B. pinnipedialis* NCTC 12890^T; 10, *B. ceti* NCTC 12891^T; 11, *O. anthropi* LMG 3331^T; 12, *O. intermedium* LMG 3301^T. oNP, *o*-Nitrophenyl; pNP, *p*-nitrophenyl; QO₂N, mean of 10 repetitions per reaction. +, Positive (QO₂N values >70); –, negative (QO₂N values <50); v, variable (QO₂N values >50 and <70).

Compound	1	2	3	4	5	6	7	8	9	10	11	12	Substrate class
L-Glutamine	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid
L-Histidine hydrochloride	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid
D-Histidine	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid
L-Citrulline	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid
DAPI	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid
Sarcosine hydrochloride	+	+	–	–	–	–	–	–	–	–	+	+	Amino acid derivative
Fumaric acid	+	+	–	–	–	–	–	–	–	–	+	+	Org. acid
Succinic acid	+	+	–	–	–	–	–	–	–	–	+	+	Org. acid
α -Ketoglutaric acid	+	+	–	–	–	–	–	–	–	–	+	+	Org. acid
Itaconic acid	+	+	–	–	–	–	–	–	–	–	–	–	Org. acid
Mesaconic acid	+	+	–	–	–	–	–	–	–	–	–	–	Org. acid
Glutaric acid	+	+	–	–	–	–	–	–	–	–	+	+	Org. acid
Arginine <i>p</i> -nitroanilide	+	+	–	–	–	–	–	–	–	–	v	+	Amino peptidase
L-Pyroglutamic acid β -naphthylamide	–	–	–	–	–	–	–	–	–	–	+	+	Amino peptidase
pNP <i>N</i> -Acetyl- β -D-galactosaminide (pH 7.5)	+	+	–	–	–	–	–	–	–	–	v	+	Esterase
oNP <i>N</i> -Acetyl- α -D-galactosaminide (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Esterase
oNP <i>N</i> -Acetyl- β -D-galactosaminide (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Esterase
pNP <i>N</i> -Acetyl- β -D-glucosaminide (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Esterase
pNP <i>N</i> -Acetyl-1-thio- β -D-glucosaminide (pH 7.5)	v	+	–	–	–	–	–	–	–	–	+	+	Esterase
4-Nitrophenyl- α -D-maltoheptaoside-4,6,0-ethylidene (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Esterase
pNP <i>N</i> -Acetyl- β -D-glucosamide (pH 5.5)	+	+	–	–	–	–	–	–	–	–	+	–	Esterase
pNP <i>N</i> - α -L-Arabinopyranoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
oNP <i>N</i> - β -D-Xylopyranoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP <i>N</i> -1-thio- β -D-Galactopyranoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
2-Methoxy-4-(2-nitrovinyl)-phenyl β -D-galactopyranoside	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP β -L-Fucopyranoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP β -D-Thiofucopyranoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP β -D-Maltoside (pH 7.5)	+	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP β -D-Lactopyranoside (pH 7.5)	v	+	–	–	–	–	–	–	–	–	+	+	Glucosidase
pNP β -D-Glucuronide (pH 5.5)	+	+	–	–	–	–	–	–	–	–	+	–	Glucosidase
Aesculin hydrolysis	–	–	–	–	v	–	v	–	–	–	+	+	Classical reaction
Voges–Proskauer reaction	+	+	–	–	–	–	–	–	–	–	+	+	Classical reaction
oNP β -D-Galactopyranoside-6-phosphate	+	+	–	–	–	–	–	–	–	–	+	+	Phosphatase
pNP Phosphate-di(2-amino-2-ethyl-1,3-propanediol) (pH 5.5)	+	+	–	–	–	–	–	–	–	–	+	+	Phosphatase

ments, multilocus sequence typing (MLST) analysis and IS711-specific PCR (De *et al.*, 2008). DNA–DNA reassociation of >70 % between strain BO1^T and the type strains of *B. melitensis* (80 %) and *B. suis* (78 %) as well as the

presence of the *Brucella*-specific IS711 element clearly demonstrated that strain BO1^T is a member of the genus *Brucella*. However, the data from 16S rRNA gene sequencing and MLST analysis also revealed that strain BO1^T was

markedly different from all recognized *Brucella* species and their biovars. For example, strain BO1^T is characterized by a unique 16S rRNA gene sequence differing in five nucleotides when compared with the 16S rRNA gene consensus sequence of *Brucella* species and also exhibits a significantly lower level of sequence similarity in various housekeeping genes (De *et al.*, 2008).

In this study, we have additionally sequenced the *recA*, *omp2a/b*, *omp25* and *omp31* genes as described for *B. microti* (Scholz *et al.*, 2008a). As shown previously, all recognized *Brucella* species, including *B. microti*, are identical in their *recA* gene sequences (Scholz *et al.*, 2008b). However, the partial *recA* gene sequence of strain BO1^T (GenBank no. FM177719) differed in seven of 631 nucleotides (98.9 % identity). These findings again emphasized the unique position of strain BO1^T within *Brucella*. As in MLST analysis, the various *omp* gene sequences of strain BO1^T also

exhibited lower sequence similarities. The *omp31* gene (723 bp) was only 96 % identical to the respective sequences of other *Brucella* species. The *omp25* gene (642 bp) sequence of strain BO1^T was most closely related to *omp25* of *B. suis* ATCC 23445 (GenBank no. CP000911) with a similarity of 99 %. In the phylogenetic analysis using the neighbour-joining method, the *omp2a* and *omp2b* gene sequences of BO1^T were most closely related to *omp2a/b* of *Brucella* strain 83-210 (83/13), an isolate with uncertain affiliation (Fig. 2), and differed considerably (97–98 % sequence similarity) from *omp* gene sequences of other *Brucella* species.

In addition to sequencing of the above-mentioned genes, strain BO1^T was characterized by using AMOS PCR (Bricker & Halling, 1994) and species-specific multiplex PCR (Garcia-Yoldi *et al.*, 2006). In multiplex PCR analysis a unique pattern of six PCR fragments (150 bp, 272 bp, 450 bp, 587 bp, 794 bp and 1682 bp), not observed in

Fig. 2. Phylogenetic tree reconstructed with *omp2a* (1104 nt) and *omp2b* (1089 nt) sequences using CLUSTREE neighbour-joining analysis (Thompson *et al.*, 1994) and Kimura distances. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets. The tree was rooted by outgrouping sequence *omp2a* of *O. anthropi* LMG 3331^T. Bar, 0.1 divergent residues per site.

other *Brucella* species, was generated. AMOS PCR was negative using all primer combinations (data not shown). Finally, strain BO1^T was typed by using IS711 fingerprinting using the Southern blotting technique and by multilocus VNTR (variable-number tandem-repeat) analysis (MLVA) using eight mini- and eight micro-satellite VNTR markers as described recently for *B. microti* (Scholz *et al.*, 2008a). Of the *Brucella*-specific insertion sequence IS711, four to five copies, displaying a unique banding pattern, were detected (Supplementary Fig. S3, in IJSEM Online). The MLVA typing pattern obtained was significantly different from those for previously analysed strains (Le Flèche *et al.*, 2006; Al Dahouk *et al.*, 2007). A new intermediate size allele was observed at locus Bruce42 (coded 2.5). No amplification product could be obtained at four loci, bruce07, bruce18, bruce21 and bruce30 (Le Flèche *et al.*, 2006), an exceptional behaviour not observed previously. All these loci are associated with an interspersed repeat element (containing one or two octameric tandem repeats) present in about 20 locations in *B. melitensis* 16M^T strain as reported previously by Bricker *et al.* (2003). This suggests that these four loci are the result of recent spreading events of this presumably mobile element within the *Brucella* genus, after the occurrence of the last common ancestor to *B. inopinata* sp. nov. and to the other *Brucellae*. Alternatively, these four loci might have been lost or rearranged along the inopinata lineage as a result of their peculiar structure. MLVA cluster analysis using the categorical distance coefficient and the unweighted pair-group method with arithmetic averages (UPGMA) was carried out as described previously (Scholz *et al.*, 2008a). In these analyses, strain BO1^T clustered together with the *B. microti*, *B. suis* biovar 5 and *B. neotomae* strains and three representative marine mammal strains (Supplementary Fig. S4, in IJSEM Online) in a very loosely connected group with long branches. The inopinata branch is in proportion markedly shorter than the relatively very long branch observed by using MLST (De *et al.*, 2008). This might be because of the inherent homoplasy at MLVA loci. Furthermore, relatively long evolutionary distances will be underestimated by MLVA as 16 markers were used in this MLVA assay, and the distance is the categorical coefficient; an MLVA distance between two strains cannot be more than 16. Another possibility is that VNTR markers do not evolve as fast in these presumably environmental species, in comparison with the highly pathogenic and host-adapted variants (the more classical *Brucellae*), which are under different stress conditions. However, as yet nothing is known about the natural reservoir or the host-range of this novel *Brucella* species.

From the phenotypic and molecular data it was obvious that strain BO1^T belonged to the genus *Brucella* but also differed from all recognized species and their biovars. We therefore conclude that BO1^T represents a novel species of the genus *Brucella*, for which the name *Brucella inopinata* sp. nov. is proposed.

Description of *Brucella inopinata* sp. nov.

Brucella inopinata (in.o.pi.na'ta. L. fem. adj. *inopinata* unexpected).

Aerobic, non-fermentative, non-motile, non-spore-forming, Gram-negative coccobacilli or short rods, 0.5–0.7 µm in diameter and 0.6–1.2 µm in length arranged individually or in irregular clusters. Non-fastidious, fast growth (visible within 6 h) occurs on *Brucella* agar, trypticase soy agar (Becton Dickinson), sheep blood agar and standard nutrient agar at 25–42 °C. Positive for growth on MacConkey agar (Becton Dickinson). Smooth, opaque colonies are formed within 18 h, with a diameter of approximately 1–2 mm. Good growth does not require CO₂, supplementary serum or blood. No haemolysis is observed. Growth occurs in the presence of thionin at concentrations of 1/25 000, 1/50 000 and 1/100 000, and with basic fuchsin at concentrations of 1/50 000 and 1/100 000. Incomplete lysis occurs with bacteriophages Tb, F1 and F25 at 10⁴ × RTD, but not at other dilutions. Agglutinates with monospecific anti-M serum up to a dilution of 1:40. Oxidase- and catalase-positive. Fast urease reaction (<5 min). Growth occurs in nutrient broth with or without 6% NaCl. Nitrate and nitrite are reduced (with gas formation from nitrate). Production of H₂S and Voges–Proskauer reaction are positive. Negative for hydrolysis of aesculin, gelatin liquefaction, production of indole, citrate utilization, growth on cetrimide and *Salmonella*–*Shigella* (SS) agar. Acid production is observed in King's oxidation–fermentation base from D-glucose and D-xylose, whereas no acid production is observed in King's oxidation–fermentation base from mannitol, lactose, sucrose and maltose. Positive (API ZYM) for acid phosphatase, alkaline phosphatase, trypsin, leucine arylamidase and naphthol-AS-BI-phosphohydrolase. Negative (API ZYM) for esterase, esterase lipase, lipase, valine arylamidase, cystine arylamidase, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Positive (API 20NE) for D-glucose, maltose, L-arabinose, D-mannose, N-acetylglucosamine and adipic acid. Negative for D-mannitol, citric acid, gluconate, capric acid, malic acid and phenylacetic acid. In API 20E, positive for fermentation of L-arabinose. Additional differential physiological reactions (MICRONAUT) of strain BO1^T in comparison with other *Brucella* species and biovars and *O. anthropi* LMG 3331^T are given in Table 1. Susceptible to the following antimicrobial agents: doxycycline (0.12 µg ml⁻¹), tetracycline (0.25 µg ml⁻¹), streptomycin (2 µg ml⁻¹), gentamicin (1 µg ml⁻¹) and trimethoprim–sulfa-methoxazole (<0.5 and 9.5 µg ml⁻¹). Major whole-cell cellular fatty acids (5–46%) are C_{19:0} cyclo 11-12, C_{18:1ω7c}, C_{18:0} and C_{16:0}, and smaller amounts (1–4%) of Br-C_{19:1}. Quinone system is composed of the major compound ubiquinone Q-10 and minor amounts of Q-11. The polar lipid profile contains the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine and one unknown

aminolipid, moderate amounts of phosphatidylmonomethylethanolamine and minor to trace amounts of an unknown aminophospholipid, an unknown phospholipid and two unknown polar lipids. The polyamine pattern consists of the major compounds spermidine and putrescine and small amounts of 1,3-diaminopropane, *sym*-homospermidine and spermine.

The type strain, BO1^T (=BCCN 09-01^T=CPAM 6436^T), was isolated in 2005 from a breast implant infection of a 71-year-old female patient in the USA.

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