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ORIGINAL PAPER

Corynebacterium ulcerans isolates from humans and dogs: fibrinogen, fibronectin and collagen-binding, antimicrobial and PFGE profiles

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Abstract *Corynebacterium ulcerans* has been increasingly isolated as an emerging zoonotic agent of diphtheria and other infections from companion animals. Since pets are able to act as symptomless carriers, it is also essential to identify virulence potential for humans of these isolates. In this work the ability of *C. ulcerans* to bind to fibrinogen (Fbg), fibronectin (Fn) and Type I collagen as well the genetic relationship among strains isolated from human and asymptomatic dogs in Rio de Janeiro (Brazil) were analyzed. Five pulsed-field gel electrophoresis (PFGE) profiles were demonstrated (I, II, III, IV and V). In addition, the IV and V profiles exhibiting ≥85 % similarity were expressed by the BR-AD41 and BR-AD61 strains from companion dogs living in the same neighborhood. Independent of the PFGE-types, human and dog isolates showed affinity to Fbg, Fn and collagen. Heterogeneity of PFGE profiles indicated endemicity of *C. ulcerans* in the Rio de Janeiro metropolitan area. Differences in the expression of adhesins to the human extracellular matrix may contribute to variations in the virulence and zoonotic potential of *C. ulcerans* strains.

Keywords *Corynebacterium ulcerans* · Dog · Fibrinogen · Fibronectin · PFGE

Introduction

Infection with *Corynebacterium ulcerans* is recognised as an emerging zoonotic disease and there is evidence that the number of cases and the severity of

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clinical signs is increasing in industrialized and developing countries (Dewinter et al. 2005; De Zoysa et al. 2005; Mattos-Guaraldi et al. 2008a; Wagner et al. 2010; Kamada et al. 2012; Urakawa et al. 2013). Human infections by *C. ulcerans* may be fatal and mostly occur in adults with close animal contact (Wellinghausen et al. 2002; Lartigue et al. 2005). Lately, *C. ulcerans* has been increasingly isolated as emerging zoonotic agent from companion animals such as cats and dogs (Dias et al. 2011a; Zakikhany and Efstratiou 2012). There is, therefore, a potentially large reservoir of infection with little knowledge about the risks of zoonotic transmission. The majority of the cases of diphtheria caused by *C. ulcerans* has occurred in adult patients who had been fully or partially immunized with diphtheria toxoid vaccine (Leek et al. 1990; Dias et al. 2011a). In Europe, *C. ulcerans* is currently isolated in more frequency from diphtheria cases than *Corynebacterium diphtheriae* (De Zoysa et al. 2005; Perkins et al. 2010; Taylor et al. 2010; Wagner et al. 2010). *C. ulcerans* does not cause large epidemics and human-to-human transmission remains uncertain (Bonnet and Begg 1999; Mattos-Guaraldi et al. 2008a).

The aim of this study was to investigate the affinity of *C. ulcerans* to human plasma fibrinogen (Fbg), fibronectin (Fn) and Type I collagen as a virulence property. In addition, the antimicrobial susceptibility profiles and the genetic relationship among *C. ulcerans* strains isolated from asymptomatic dogs and a human patient in Rio de Janeiro metropolitan area, Brazil were also determined.

Materials and methods

Origin of bacterial strains and culture conditions

Three *C. ulcerans* strains isolated from asymptomatic dogs and one from human patient in Rio de Janeiro metropolitan area, during the period of 2000–2012, were analyzed (Table 1): BR-AD41 and BR-AD61 strains currently isolated, respectively, from nares to skin wounds of two domestic dogs in Duque de Caxias city; BR-AD22 strain, previously isolated from nares of a shelter dog in Niterói city (Dias et al. 2010); 809 strain previously isolated from an elderly woman with a fatal pulmonary infection and history of leg skin ulcers in the Rio de Janeiro city (Mattos-Guaraldi et al. 2008a). *C. ulcerans* CDC KC279 strain, *Corynebacterium pseudotuberculosis* 1002 strain; *C. diphtheriae* subsp. *mitis* ATCC 27010 (C7 s (−) tox− [NCTC 11397]) and ATCC 27012 strains were also analyzed for comparison.

To rule out the presence of any infectious or debilitating diseases, including dermatoses and the more obvious physical and behavioral abnormalities, a general assessment of each animal's condition and nutritional status was recorded as previously described (CCAC 2013). Swabs from skin lesions, nares and ears samples from companion to shelter dogs were collected with sterile swabs and inoculated onto the chocolate tellurite agar plates and incubated at 35–37 °C for 72 h as described by Dias et al. (2011b). Stock cultures in 10 % skim milk with 25 % added glycerol were maintained at −70 °C and recovered as required by cultivation in Trypticase Soy Agar (TSA; Difco Laboratories, Detroit, MI.) at 37 °C for 24 h under aerobic conditions (Dias et al. 2010).

Phenotyping, molecular characterization and toxigenicity testing

The identification of *C. ulcerans* strains was achieved using both phenotypic and genotypic methods. Positive bacterial cultures for irregular Gram-positive rods were preliminarily characterized by colonial morphology, pigmentation, hemolysis and DNAse activity. The suspect bacterial isolates were identified as *C. ulcerans* by conventional biochemical assays and the semi-automatized API-Coryne System V3.0 (bioMérrieux, Lyon, France) with the API web decoding system http://www.apiweb.biomerieux.com (Efstratiou and George 1999; Funke and Bernard 2007; Pimenta et al. 2008; Dias et al. 2010). The production of phospholipase D (Pld) was evaluated by the CAMP test (i.e., inhibition of hemolysis by *Staphylococcus aureus*).

Molecular identification of the isolates was carried out by a multiplex PCR (mPCR) used to provide simultaneous identification and toxigenicity of these corynebacterial species with zoonotic potential in addition to *C. diphtheriae*, based on primers targeting the following genes: *rpoB* (*Corynebacterium* spp.), *16S rRNA* (*C. ulcerans* and *C. pseudotuberculosis*), *pld* (specific for *C. pseudotuberculosis*), *dtxR* (*C. diphtheriae*) and *tox* gene (diphtheria toxin-DT) (Torres et al. 2013).

Table 1 *Corynebacterium ulcerans* strains isolated from human and asymptomatic dogs in Rio de Janeiro metropolitan area, Brazil

C. ulcerans isolates	City/year	Source	Clinical detail	API-Coryne code (probability, T = 0.99)	References
Human					
809	Rio de Janeiro/2000	Lung	Fatal pulmonary infection	0111326 (99.7 %)	Mattos-Guaraldi et al. (2008a), Trost et al. (2011)
Dog shelter					
BR-AD22	Niterói/2010	Nare	Asymptomatic	0111324 (7.2 %)	Dias et al. (2010), Trost et al. (2011)
Companion					
BR-AD41	Duque de Caxias/2012	Tick bite skin lesion	Asymptomatic	0111326 (99.7 %)	This study
BR-AD61	Duque de Caxias/2012	Nare	Asymptomatic	0111306 (96.6 %)	This study

RJ Rio de Janeiro, *DC* Duque de Caxias, *N* Niterói

Antimicrobial susceptibility profiles

The sensitivity to antimicrobial agents (Oxoid, Hampshire, UK), penicillin G (10 U), ampicillin (10 μg), cefotaxime (30 μg), imipenem (10 μg), erythromycin (15 μg), clindamycin (2 μg), tetracycline (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), rifampicin (5 μg), linezolid (30 μg) and vancomycin (30 μg) was determined by the disk diffusion method using inoculum equivalent to a 0.5 McFarland standard, according to previously adopted by other authors (Martinez-Martinez et al. 1995; CLSI 2007; Dias et al. 2010). Plates were incubated at 37 °C for 24 h and reconfirmed at 48 h using a cation-adjusted Mueller–Hinton agar with 5 % sheep blood. Breakpoints for the susceptible strains were used as suggested by the CLSI for bacteria excluded from table 2A to 2K. As there is not yet a defined standard for interpreting these results, the one proposed in the CLSI document M45-A (ISBN 1-56238-607-7) was used (CLSI 2007). The breakpoints for *S. aureus* established by CLSI were considered in the cases of penicillin and ampicillin. Minimum inhibitory concentrations (MIC) of penicillin G and clindamycin were also evaluated by the *E*-test (AB Biodisk, Solna, Sweden), as previously described (Martinez-Martinez et al. 1995).

ECM and plasma proteins binding assays

Bacterial binding assays using biotinylated Fbg, Fn and Type I collagen (all from Sigma Chemical Co.) were performed in 96 wells Elisa Microtiter plates (Costar 96 Well EIA/RIA Plate; Corning, NY, USA). Bacterial cultures grown for 24 h at 37 °C in trypticase soy broth—TSB medium were washed 2× with phosphate buffered saline (PBS), and resuspended in 0.1 M $NaHCO_3$, pH 9.6 to a suspension of OD 0.2 at $\lambda = 650$ nm (equivalent to 5×10^9 CFU/mL). The wells were sensitised with 100 μL of bacterial suspensions for 1 h at 37 °C, and overnight at 8 °C. A standard curve was also performed by diluting the biotinylated protein solutions to concentrations varying from 5 to 0.05 μg (1 h/37 °C). After blocking with 2 % bovine serum albumin (BSA Type V, Sigma) in PBS added of 0.05 % Tween-20 (PBST) for 1 h at 37 °C, the wells were washed 3× with PBST. The bacterial strains were reacted with 20 μg/mL of biotinylated ECM/plasma proteins for 1 h at 37 °C. After washing 3× with PBST, the wells were reacted for 30 min at 37 °C with 0.001 μg/mL Extravidin-peroxidase (Sigma) prepared in PBST 1 % BSA. After washing 3× with PBST, the reaction was verified by adding 3,3′,5,5′-tetramethylbenzidine liquid substrate (TMB, Sigma) for 20 min and the reaction blocked with 50 μL of 1 M HCl. The reaction was read in $\lambda = 450$ nm in a microtiter plate reader. The colour intensity of the wells sensitised with the microorganisms was compared to the standard curve by GraphPad Prism 6.0 version. The results were expressed in micrograms of adhered proteins, in a mean ± SD of three independent assays performed in triplicate. The mean of the binding properties were compared by

Tukey's multiple comparison test (Harlow and Lane 1988; Sabbadini et al. 2010).

Pulsed-field gel electrophoresis (PFGE)

Genomic DNA was prepared following a method described previously (Baio et al. 2013). The DNA was cleaved with *Sfi*I (New England BioLabs) according to the manufacturer's instructions. PFGE was carried out in 0.5× TRIS–borate–EDTA–1.2 % agarose gels at 13 °C with a CHEF DRII system (Bio-Rad). The pulse times were 1–30 s over 20 h. A lambda DNA concatemer (New England BioLabs) was used as a molecular size marker. Similarities among macrorestriction patterns were identified according to the criteria established by Tenover et al. (1995). The BioNumerics Fingerprinting software (Version 4.0, Applied Math, Sint-Martins-Latem, Belgium) was used to confirm the findings provided by visual observation. The similarity index of the strains was calculated using the Dice correlation coefficient with a band position tolerance of 1 % and unweighted-pair group method using average linkages (UPGMA) was used to construct a dendrogram. Strains were considered to belong to the same PFGE group if the similarity index was ≥85 % percentage band-based similarity coefficients as cut-off values.

Ethical procedures

The study was performed in compliance with the guidelines outlined in the Canadian Council on Animal Care (CCAC) and with the Brazilian government's ethical guidelines for research involving animals (Fiocruz Ethic Committee for Animal Experiments-CEUA/FIOCRUZ-LW-64/12).

Results

Corynebacterium ulcerans phenotypic and genotypic characterization

C. ulcerans strains were positive for catalase, urease, alkaline phosphatase and α-glucosidase. Nitrate reduction, pyrazinamidase, gelatinase, and esculin hydrolysis tests gave negative results. Fermentation tests were positive for glucose, ribose; negative for xylose, mannose, sucrose and lactose. Maltose and glycogen fermentation were variable characteristics. Pld production was demonstrated by the positive reverse CAMP test. Amplification profiles by mPCR identified BR-AD22, BR-AD41 BR-AD61 and 809 Brazilian strains as *C. ulcerans* (Fig. 1) with negative results for *tox* gene.

Antimicrobial susceptibility profiles

C. ulcerans BR-AD22, BR-AD41, BR-AD61 and 809 strains showed moderate susceptibility to penicillin G and clindamycin (Table 2). All *C. ulcerans* strains tested showed susceptibility to other antimicrobial agents tested, including erythromycin and vancomycin.

ECM/plasma proteins binding properties

The ability of *C. ulcerans* to bind to human Fbg, Fn and Type I collagen molecules was demonstrated at varied levels (Fig. 2; Table 2). The three dog isolates (BR-AD22, BR-AD41, BR-AD61 strains) were capable to binding over than 10 % of biotinylated proteins, when reacted with 100 µL of a solution containing 20 µg/mL for 1 h/37 °C. Data showed a higher affinity of the dog isolates BR-AD22, BR-AD41, BR-AD61 strains to Fbg while a higher affinity of BR-AD22 and BR-AD61 strains to Fn; and higher affinity of BR-AD22 to Type I collagen was shown. ELISA results showed the highest affinity to Fbg, Fn and Type I collagen for BR-AD22 strain ($P < 0.001$). Conversely, 809 strain (isolated from a human patient with lethal pneumonia) adhered to human ECM/plasma proteins in lower intensities ($P < 0.001$). CDC KC279 strain adhered to collagen and Fn in similar intensities of BR-AD61 and BR-AD41 strains, respectively ($P > 0.05$). BR-AD41 and BR-AD61 showed similar adherence levels to Fbg ($P > 0.05$).

PFGE analysis

PFGE of the *Sfi*I-digested DNA of 5 strains revealed distinct PFGE profiles which were designated I, II, III, IV and V (Fig. 3). The PFGE profile IV differed by more than three bands from PFGE profile V by visual inspection. According to the interpretation criteria by Tenover et al. (1995), the BR-AD41 strain belonging to profile IV and BR-AD61 strain, profile V were considered related. These strains were isolated from

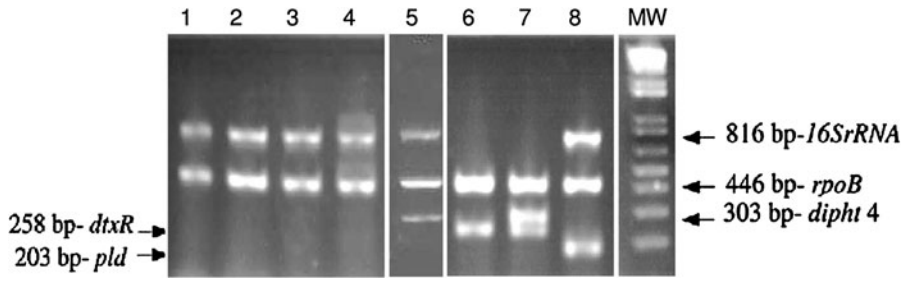

Fig. 1 Amplification profile by multiplex polymerase chain reaction of *Corynebacterium ulcerans* (*lanes 1–5*), *Corynebacterium diphtheriae* (*lanes 6 and 7*), *Corynebacterium pseudotuberculosis* (*lane 8*): *lane 1* BR-AD22 (tox−); *2* BR-AD41 (tox−); *3* BR-AD61 (tox−); *4* 809; *5* CDC KC279 (tox+); *6* ATCC 27010 (tox−); *7* ATCC 27012 (tox+); *8* 1002 (tox−); *9* molecular weight (100-bp DNA ladder)

Table 2 Phenotypic and genotypic characteristics of *Corynebacterium ulcerans* strains isolated from human and dogs in Rio de Janeiro metropolitan area, Brazil (2000–2012)

Strain number	Antimicrobial agents Moderate susceptibility to[b]	*tox* gene/ Pld/ DNAse	Binding (μg)[c]			PFGE type
			Fbg	Fn	Collagen	
CDC KC 279[a]	Penicillin G (MIC 0.19 ≥ mg/L); clindamycin (MIC ≥ 1.5 mg/L)	+/+/+	0.2000 ± 0.0005774	0.4067 ± 0.006642	0.4680 ± 0.01328	I
809	Penicillin G, clindamycin	−/+/+	0.1270 ± 0.003464	0.1367 ± 0.007219	0.1650 ± 0.02801	II
BR-AD22	Penicillin G, clindamycin	−/+/+	0.2690 ± 0.004041	0.7160 ± 0.004619	0.1650 ± 0.02801	III
BR-AD41	Penicillin G, clindamycin	−/+/+	0.2290 ± 0.008660	0.3937 ± 0.001453	0.3280 ± 0.004041	IV[d]
BR-AD61	Penicillin G, clindamycin	−/+/+	0.2370 ± 0.0005774	0.6200 ± 0.001386	0.4557 ± 0.005487	V[d]

NI not informed, *PLD* phospholipase D, *DNAse* deoxyribonuclease, *Fbg* fibrinogen, *Fn* fibronectin, + positive, − negative

[a] Control strain

[b] Evaluated by disk diffusion method and *E*-test

[c] Results expressed as a mean of three independent assays performed in triplicate

[d] PFGE-types epidemiologically related

dogs in the same neighborhood and exhibited ≥85 % similarity, so they were considered as belonging to the same PFGE group. The other PFGE profiles exhibited more than six bands of difference, indicating that they were epidemiologically unrelated.

Discussion

Toxigenic *C. ulcerans* and *C. diphtheriae* can lead to life-threatening disease that requires urgent treatment with diphtheria antitoxin (DAT) without waiting for laboratory confirmation. Antibiotic treatment of diphtheria-like illness caused by *C. ulcerans* should follow clinical guidelines for patients infected with *C. diphtheriae*. An appropriate antibiotic (penicillin or erythromycin) should be used to eliminate the causative organisms, stop exotoxin production and reduce communicability (Pickering et al. 2009; CDC 2011, 2012; Sekizuka et al. 2012). Clindamycin was formerly considered as an alternative in the treatment of the carrier of diphtheria bacilli, especially in those who were sensitive to penicillin (Zamiri and McEntegar 1972).

Similar PFGE profiles or ribotypes have been previously observed in *C. ulcerans* strains isolated from human and animal colonization or infectious processes (Katsukawa et al. 2009, 2012; Komiya et al.

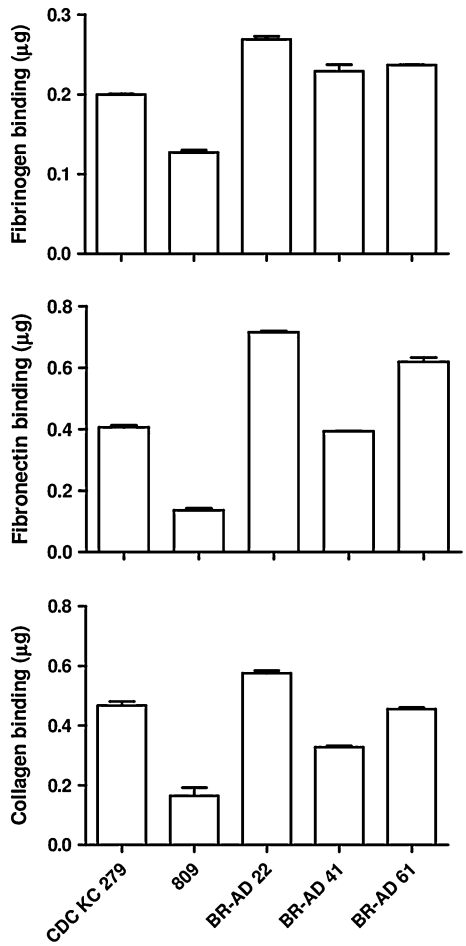

Fig. 2 Binding to human fibrinogen (Fbg), fibronectin (Fn) and Type I collagen by *Corynebacterium ulcerans* evaluated by enzyme linked immunosorbent assay (ELISA): BR-AD22, BR-AD41 and BR-AD61 strains isolated from dogs, 809 strain from human; control CDC KC279 strain. Data showed the highest affinity to Fbg, Fn and Type I collagen for BR-AD22 strain ($P < 0.001$). The results were expressed in mean ± SD of three independent assays performed in triplicate. The mean of the binding properties were compared by Tukey's multiple comparison test

2010; Berger et al. 2011). In Japan, 45 *C. ulcerans* dog isolates including 39 strains of a predominant PFGE type (A2), showed resistance or decreased sensitivity to clindamycin. The authors suggested that transmission among asymptomatic dogs might have occurred (Katsukawa et al. 2012). In our study, heterogeneity of PFGE profiles observed in strains isolated from human and dogs indicated endemicity of *C. ulcerans* in Rio de Janeiro metropolitan area. *C. ulcerans* strains of five different PFGE types showed moderate susceptibility to penicillin G and clindamycin, as previously demonstrated for other Brazilian *C. diphtheriae* strains (Pereira et al. 2008). Interestingly, two epidemiologically related PFGE profiles (profiles IV and V) were found in the BR-AD41 and BR-AD61 dog isolates from Duque de Caxias city in 2012.

Virulence mechanisms of *C. ulcerans* should become a matter of higher interest especially due to the increase in the number and severity of cases of infection in immunized or partially immunized individuals. *C. ulcerans* strains seem to be endowed with an array of virulence factors other than DT such as catalase, proteases, deoxyribonuclease (DNAse), neuraminidase H (NanH), endoglycosidase E (EndoE), Pld toxin and subunits of adhesive pili of the SpaDEF type (Trost et al. 2011).

Corynebacterium ulcerans strains producing Pld, but not DT toxin, are able to cause severe disease in humans, such as lymphadenitis, dermatitis, subcutaneous abscess and acute pharyngitis and lower respiratory tract infections (pneumonia and granulomatous nodules in pulmonary tissues) (Desseau et al. 1995; Hommez et al. 1999; Hatanaka et al. 2003; Dias et al. 2011a). Consequently, *C. ulcerans* strains unable to produce DT toxin should not be underestimated. Pld may cause an increase in vascular permeability, has dermonecrotic properties, and reduces viability of neutrophils and macrophages (Schmiel and Miller 1999). In an attempt to further investigate possible mechanisms that promote *C. ulcerans* infection and hematogenic dissemination, a previous study revealed a strain-dependent arthritogenic potential independent of catalase, DNAse, Pld and DT production. Some *C. ulcerans* strains showed a higher arthritogenic and mortality potential when compared to *C. diphtheriae* strains during an in vivo experimental infection in mice. *C. ulcerans* arthritis also had a hematogenic spread and viable bacteria were recovered from joints, blood, kidneys, liver, and spleen but not from the heart and lungs of mice (Gaede and Heesemann 1995; Tissi et al. 1999; Puliti et al. 2006; Dias et al. 2011b). *C. ulcerans* 809 human isolate (*tox* gene negative) caused skin lesions with a large extent of yellowish-white fibrinous (fibrin) deposits in the lower limb of the patient (Mattos-Guaraldi et al. 2008a). Dermonecrotic activity was also observed for both human 809 and dog BR-AD22 isolates in experiments performed with guinea pigs (Mattos-Guaraldi et al. 2008b).

Comparative analysis of the complete genomes of *C. ulcerans* 809 and BR-AD22 strains and detection of

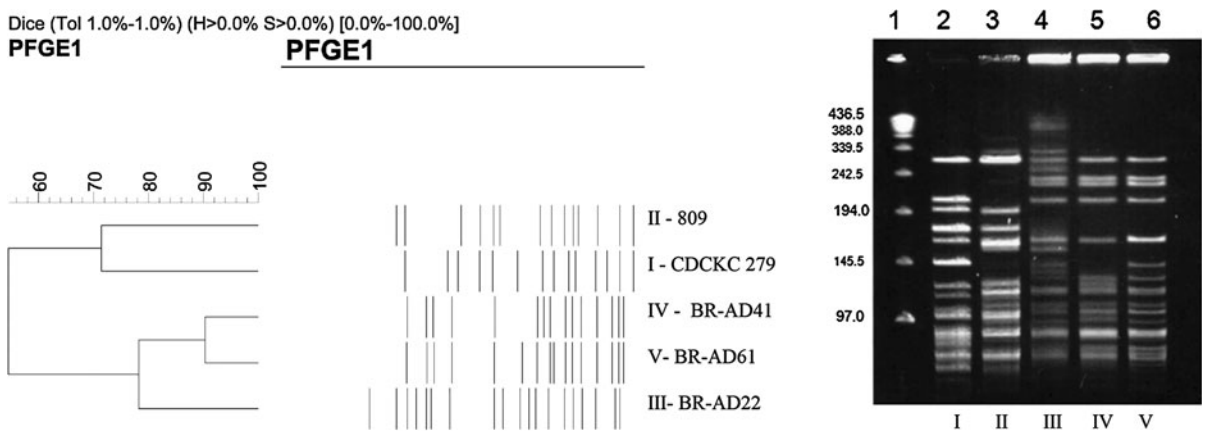

Fig. 3 Pulsed-field gel electrophoresis (PFGE) profiles of Brazilian *Corynebacterium ulcerans* strains isolated from human and dogs. **a** Dendrogram of results of PFGE data. The percent similarity scale is based on UPGMA clustering of Dice coefficients generated by BioNumerics software v 4.0; **b** PFGE assay. *Lane 1* λ DNA ladder PFGE marker; *lane 2* CDC KC 279 control strain (USA), profile I; *lane 3* 809 (human), profile II; *lane 4* BR-AD22 (dog), profile III; *lane 5* BR- AD41 (dog), profile IV; *lane 6* BR-AD61 (dog), profile V

candidate virulence factors was recently performed (Trost et al. 2011). The detection and functional assignments of singletons confirmed that the repertoire of potential virulence factors of the sequenced *C. ulcerans* were different in the two selected isolates from a human and animal source (809 and BR-AD22 strains, respectively). Two genes encoding surface-anchored proteins with LPXTG motif, including the *SPAD* gene for the major pilin subunit of an adhesive pilus structure, were also detected as singletons. SpaD protein of *C. ulcerans* BR-AD22 differs in its amino acid sequence when compared with the functional counterpart CULC809-01952 from *C.ulcerans* 809, demonstrated that pili of the two *C. ulcerans* strains varied significantly in the primary sequence of their major pilins that in principle constitute the shaft of the corynebacterial pilus structure. Data suggested differences in the adhesive properties of *C. ulcerans* strains.

The first step in the infectious process of extracellular pathogens like *C. diphtheriae* is generally considered to be attachment to and colonization of host tissue surfaces. Evidence from other Gram-positive pathogens suggests that bacterial surface adhesins recognizing adhesive matrix molecules or plasma proteins may serve as potential antigenic candidates for the development of novel immunotherapies, including diphtheria-like illness and invasive infections caused by *C. ulcerans* (Rivera et al. 2007).

Fbg is a major protein in human plasma that has its synthesis dramatically upregulated during inflammation or under exposure to stress such systemic infections. It is, therefore, not surprising that many bacterial pathogens can interact with Fbg and manipulate its biology (Rivera et al. 2007). Fbg is primarily involved in the coagulation cascade system through its conversion to insoluble fibrin. Both Fbg and fibrin play overlapping roles in blood clotting, fibrinolysis, inflammatory response, cellular and matrix interactions and wound healing. Many bacterial pathogens exploit mechanisms involved in coagulation systems to colonize exposed tissue matrix proteins or evade immune mechanisms of bacterial clearance (Doolittle 1984; Lantz et al. 1985; Mosesson 2005; Sun 2006). The presence of Fbg onto bacterial surfaces may be an efficient trait to avoid phagocytosis in human hosts, as previously described with other Gram-positive pathogens (Schubert et al. 2002; Rennermalm et al. 2004; Pierno et al. 2006), including *C. diphtheriae* (Gomes et al. 2009; Sabbadini et al. 2010).

Fn is a complex glycoprotein found in a soluble form in many body fluids (blood, saliva) and in an insoluble form as a component of cell surfaces, basement membranes, and the extracellular matrices. Soluble plasma Fn interacts with various bacteria and cell surfaces. Fn may serve as a receptor in the adherence of bacteria to host epithelial cell and may play an important role in tissue tropism. Fn can simultaneously bind to Fbg, fibrin, collagen, human cells and bacteria (Mosher 1975; Ruoslahti and Vaheri 1975; Engvall and Ruoslahti 1977; Engvall et al. 1978;

Livornese and Korzeniowski 1992). Type I collagen is the most prevalent form of several distinct types of collagen observed in the arterial walls, bone, dentin, dermis, tendon, and uterine wall. All types of collagen are active in Fn binding, but to a different degrees (Engvall et al. 1978). The binding to collagen and Fbg is mediated by the same binding site in Fn.

The ability of all five PFGE-types of *C. ulcerans* to bind to human Fbg, Fn and Type I collagen molecules was demonstrated, but at varied levels. Similar to *C. diphtheriae,* qualitative and quantitative differences in the expression of Fbg, Fn and Type I collagen-binding adhesins may contribute to variations in the virulence potential to the human host and pseudomembrane formation by *C. ulcerans* strains (Sabbadini et al. 2010). The fact that the animal isolates adhered to human ECM/plasma proteins in higher intensities indicated that some *C. ulcerans* strains have evolved mechanisms to target human defense, resulting in a microbial invasion of cells constitutive of host barriers, disruption of barrier integrity, and systemic dissemination and invasion of deeper tissues.

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Conflict of interest The authors declare that they no have conflict of interest.

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