NOTE

Phylogenetic evidence for reclassification of *Calymmatobacterium granulomatis* as *Klebsiella granulomatis* comb. nov.

Jenny S. Carter,^{1,2} Francis J. Bowden,⁴ Ivan Bastian,³ Garry M. Myers,¹ K. S. Sriprakash¹ and David J. Kemp¹

Author for correspondence: David J. Kemp. Tel: +618 8922 8412. Fax: +618 8927 5187.
e-mail: dave@menzies.su.edu.au

¹ Menzies School of Health Research, Darwin, Australia

² Centre for Indigenous Natural and Cultural Resource Management, Faculty of Aboriginal and Torres Strait Islander Studies, Northern Territory University, Darwin, Australia

³ Institute of Medical and Veterinary Science, Adelaide, Australia

⁴ AIDS/STD Unit, Royal Darwin Hospital, Darwin, Australia

By sequencing a total of 2089 bp of the 16S rRNA and *phoE* genes it was demonstrated that *Calymmatobacterium granulomatis* (the causative organism of donovanosis) shows a high level of identity with *Klebsiella* species pathogenic to humans (*Klebsiella pneumoniae*, *Klebsiella rhinoscleromatis*). It is proposed that *C. granulomatis* should be reclassified as *Klebsiella granulomatis* comb. nov. An emended description of the genus *Klebsiella* is given.

Keywords: *Calymmatobacterium*, *Klebsiella*, sequence data, phylogenetic inferences

Calymmatobacterium granulomatis is the presumed causative agent of donovanosis, an important cause of genital ulceration that occurs in small endemic foci in all continents except Europe and Antarctica. The name *C. granulomatis* was originally given to the pleomorphic bacterium cultured from donovanosis lesions by Aragão & Vianna (1913). Although these early cultures are highly dubious and were probably not of the organism itself (Richens, 1985), the name *C. granulomatis* has retained precedence over others.

C. granulomatis is known to be an encapsulated, non-motile, facultatively anaerobic, Gram-negative bacterium (Chandra & Jain, 1991; Davis, 1970; Davis & Collins, 1969; Dodson *et al.*, 1974; Kuberski *et al.*, 1980). *C. granulomatis* has been associated with the genus *Klebsiella* because of the above characteristics and common antigenicity (Maddocks *et al.*, 1975; Packer & Goldberg, 1950). Furthermore, two members of the genus *Klebsiella* that produce clinical disease and pathologic changes very similar to those of *C. granulomatis* are *Klebsiella rhinoscleromatis* (Levine & Hoyt, 1947; Shaw & Martin, 1961; Welsh *et al.*, 1963), which is implicated in a granulomatous disease of the nose, and *Klebsiella ozaenae* (Richens, 1985) which is implicated in chronic atrophic rhinitis. Richens (1985) goes so far as to place *C. granulomatis* in the genus *Klebsiella*, although difficulties in culturing the or-

ganism (Richens, 1991) have prevented further characterization of this relationship.

Non-cultivable pathogenic eubacteria have been identified by PCR using primers targeting conserved genes (Fredricks & Relman, 1996). We have shown through sequencing a 334 bp region of the phosphate porin (*phoE*) gene that *C. granulomatis* has a high degree of molecular identity with other *Klebsiella* species in this region (Bastian & Bowden, 1996). We present here an analysis of the almost complete 16S rRNA and *phoE* sequences for *C. granulomatis*, *K. rhinoscleromatis* and *Klebsiella pneumoniae*.

At least two punch biopsies or swabs were obtained from the lesions of eight patients with clinical donovanosis. One punch biopsy was fixed and examined for Donovan bodies by the slow Giemsa technique (Sehgal & Jain, 1987) whilst DNA was extracted from the second punch biopsy (seven samples) (Mullenbach *et al.*, 1989) or swab (one sample) (Beige *et al.*, 1995). DNA was also obtained from type cultures of *K. pneumoniae* [NCTC 9633^T (National Collection of Type Cultures)] and *K. rhinoscleromatis* (NCTC 5046^T).

Primer sets used in this study include (5'-ACCTA-CCGCAACACCGACTTCTTCGG-3' and 5'-TGA-TCAGAACTGGTAGGTCAT-3', 604 bp *phoE* prod-

Table 1. Strain designations and accession numbers for sequences obtained from the GenBank and EMBL databases, and for sequences novel to this study

Abbreviations: NCTC, National Collection of Type Cultures; ATCC, American Type Culture Collection; DSM, DMSZ – Deutsche Sammlung für Mikroorganismen und Zellkulturen; JCM, Japanese Collection of Microorganisms; PAH, Princess Alexandra Hospital, Brisbane, Australia; ^T, type Culture.

Organism	16S rRNA		<i>phoE</i>	
	Strain	Accession	Strain	Accession
<i>Calymmatobacterium granulomatis</i>	*	AF009171†	*	AF009231†
<i>Klebsiella rhinoscleromatis</i>	NCTC 5046 ^T	AF009169†	NCTC 5046 ^T	AF009230†
<i>Klebsiella pneumoniae</i>	DSM 30104 ^T	X87276	NCTC 9633 ^T	AF064793†
<i>Klebsiella oxytoca</i>	JCM 1665	AB004754	K26	X68022
<i>Klebsiella ornithinolytica</i>	JCM 7251	AB004756		
<i>Klebsiella planticola</i>	DSM 3069 ^T	X93215		
<i>Enterobacter cloacae</i>			‡	A04378
<i>Enterobacter aerogenes</i>	JCM 1235	AB004750		
<i>Citrobacter freundii</i>	ATCC 29935	M59291	‡	X68021
<i>Escherichia coli</i>	ATCC 43895	Z83205	K12	V00316
<i>Serratia marcescens</i>	ATCC 13880	M59160	UOC-51	U81967
<i>Salmonella typhi</i>	St111	U88545	‡	X74595
<i>Yersinia enterocolitica</i>	PAH11472	X68674		

* Sequences obtained from clinical specimens.

† Sequences novel to this study.

‡ Strain not specified in GenBank report.

uct); (5'-ATGAAAAAGAGTACTCTGGCA-3' and 5'-CAGACCGAAGTCGAAGTACTG-3', 840 bp *phoE* product); (5'-CCTAACACATGCAAGTCGA-3' and 5'-ATCCGACTTGACAGACCG-3', 538 bp 16S rRNA product); (5'-CGGTCTGTCAAGTCGGAT-3' and 5'-TGTAGCACGTGTGTA-GCC-3', 653 bp 16S rRNA product).

One microlitre of template DNA was included in a 25 µl PCR mixture that comprised the following solution: 10 mM Tris/HCl pH 8.0, 50 mM KCl, 2.5 mM MgCl₂, 0.01 % gelatin, 0.2 µM dNTPs, 0.5 µM primers and 0.5 Units *Taq* polymerase. Samples were subjected to 30 cycles of denaturation (94 °C, 40 s), annealing (55 °C, 40 s for *phoE* primers; 52 °C, 40 s for 16S primers) and extension (72 °C, 60 s) in a thermal cycler (Corbett Research, Sydney). All steps of PCR template preparation, PCR mix and gel analysis were conducted in three separate dedicated workrooms. Template negative controls were included in each reaction. Products of the expected size were purified and sequenced in both directions using either the dye terminator method (Applied Biosystems) or a manual T7 sequencing kit (Pharmacia-Biotech).

Initially, a 604 bp region of *phoE* was amplified and sequenced from 4/4 patients with histologically confirmed donovanosis and from 4/4 patients with negative histology for whom donovanosis remained the clinical diagnosis. The same *Klebsiella*-like sequence was amplified from all eight patients thereby con-

firmed and extending the results obtained previously (Bastian & Bowden, 1996).

In order to further investigate this relationship we determined the sequence of a coding region spanning 95 % of the *phoE* gene (1001/1059 bp) from *K. pneumoniae*, *K. rhinoscleromatis* and *C. granulomatis* isolates, along with sequences for a 1088 bp region of the 16S rRNA from *C. granulomatis* and *K. rhinoscleromatis*. The 16S rRNA sequence of *K. pneumoniae* [DSM 30104^T (DSMZ, Deutsche Sammlung für Mikroorganismen und Zellkulturen)] was obtained from the GenBank database. These sequences were aligned with the sequences of other *Klebsiella* sp. and related *Enterobacteriaceae* available from the GenBank/EMBL databases using the program CLUSTAL W (Thompson *et al.*, 1994). Due to the limited amount of complete *phoE* sequence data available, *Klebsiella planticola*, *Klebsiella ornithinolytica* and *Yersinia enterocolitica* were only included in the 16S rRNA alignment. For similar reasons different species of *Enterobacter* have been compared for the 16S rRNA and *phoE* genes.

Phylogenetic analyses was performed using the PHYLIP software package (Felsenstein, 1993). Programs employed included SEQBOOT: to produce multiple data sets by bootstrap resampling (100 iterations); DNADIST: to compute distances between species for all data sets with the Jukes-Cantor algorithm; NEIGHBOR: to produce 1000 trees (100 data sets × 10 jumbled input

Fig. 1. Phylogenetic trees showing the relationship of *C. granulomatis*, members of the genus *Klebsiella* and some related members of *Enterobacteriaceae*. Bootstrap values, expressed as percentages, are given at branch points. *Yersinia enterocolitica* and *Serratia marcescens* were used as the outgroups for 16S rRNA and *phoE* analysis respectively: (a) 16S rRNA, comparison of 1088 bp; (b) *phoE*, comparison of 1001 bp. All GenBank/EMBL accession numbers and strain designations are shown in Table 1.

orders) with the neighbour-joining method; CONSENSE: to compute the strict consensus tree by the majority-rule consensus tree method.

Accession numbers for sequences obtained from the GenBank/EMBL databases and for sequences novel

to this study are provided in Table 1 with the strain designations. Phylogenetic trees, including the confidence values of branching are shown as cladograms in Fig. 1 for the 16S rRNA and *phoE*. It is evident from tree topologies that *C. granulomatis* has a close phylogenetic relationship with members of the *Klebsiella* genus known to be human pathogens (*K. rhinoscleromatis*, *K. pneumoniae*). Bootstrap analysis data confirmed that this association was highly significant for both the *phoE* and the 16S rRNA genes (bootstrap values 100%). Nucleotide similarities between the three isolates ranged from 98.8–99.8% for the 16S rRNA and 99.7–99.8% for *phoE*.

PCR-based identification of non-cultivable microbial pathogens may be confounded by the incidental amplification of colonizing non-pathogenic bacteria. Fredricks & Relman (1996) have proposed several criteria for establishing microbial disease causation by molecular methods. Their guidelines stipulate that: the sequence-based identification should be reproducible; the nucleic acid sequence should be found at its highest concentration in diseased tissue but be absent, or present at only low copy numbers, in normal tissue; the relationship should be biologically plausible with the known phenotypic characteristics of the non-cultivable organism; and ideally the molecular identification should be established at the cellular level by *in situ* hybridization.

The studies described in this and our other papers (Bastian & Bowden, 1996; Carter *et al.*, 1997, 1999) fulfil these criteria for associating the causative organism of donovanosis with the genus *Klebsiella*. In this paper, the clinical samples from all eight patients with clinical donovanosis produced PCR products that demonstrated greater than 99% similarity with *K. pneumoniae* and *K. rhinoscleromatis* (i.e. the association is reproducible). We have developed a diagnostic PCR based on the observation that two unique base changes in the *phoE* gene of *C. granulomatis* eliminate *Hae*III restriction sites (Carter *et al.*, 1999). All 14 clinical donovanosis samples tested with this diagnostic method gave the restriction digest profile expected from sequence data. No products were obtained from patients with unrelated genital conditions (i.e. the molecular identification is specific). As described earlier, this molecular association of *C. granulomatis* with the *Klebsiella* genus is biologically plausible based on the known phenotypic and antigenic characteristics of the organism. Finally, though *in situ* hybridization experiments have not been pursued to demonstrate a tissue–sequence correlation at the cellular level, we have recently been able to cultivate *C. granulomatis* in a human epithelial cell line. DNA obtained from the cultured organisms had an identical *phoE* sequence to that obtained from clinical specimens (Carter *et al.*, 1997).

We believe that in light of our data it is reasonable to consider reclassifying *C. granulomatis* as *Klebsiella granulomatis*.

Emended description of the genus *Klebsiella*

Klebsiella species are facultatively anaerobic, Gram-negative, non-motile, generally straight rods arranged singly, in pairs or in short chains and measuring 0.3–1.0 µm in diameter and 0.6–6.0 µm in length (Ørskov, 1984). *K. granulomatis* is pleomorphic (Dienst & Bronwell, 1984), i.e. curved or straight rods, coccoid, diplococcoid, ovoid or elliptical in shape. Most *Klebsiella* species are capsulated (Ørskov, 1984) though *K. granulomatis* may be capsulated (mature form) or non-capsulated (immature form) (Hart, 1997). Most species are cultivable on routine microbiological media (Ørskov, 1984). *K. granulomatis* is facultatively intracellular, residing within the cytoplasm of large mononuclear cells and cannot be cultured on routine microbiological media (Dienst & Bronwell, 1984). There are currently five recognized species of *Klebsiella*: *K. pneumoniae*, *Klebsiella oxytoca*, *Klebsiella terrigena*, *Klebsiella planticola* and *Klebsiella ornithinolytica* (Ørskov, 1984; Sakazaki *et al.*, 1989) and three recognized subspecies of *K. pneumoniae*: *K. pneumoniae* subsp. *pneumoniae*, *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis* (Ørskov, 1984). In the absence of DNA–DNA hybridization studies it is not possible to determine whether *K. granulomatis* is a sixth *Klebsiella* species or a fourth subspecies of *K. pneumoniae*. For a complete description of the characteristics of the genus *Klebsiella* see Ørskov (1984).

Description of *Klebsiella granulomatis* comb. nov.

Klebsiella granulomatis (gran.u.lo'ma.tis. L. dim. n. *granulum* a small grain; Gr. suff. *-oma* a swelling or tumour; M.L. n. *granuloma* a granuloma; M.L. gen. n. *granulomatis* of a granuloma).

Cell characteristics. Gram-negative (Dienst & Bronwell, 1984). Non-sporulating (Richens, 1985). Non-motile (Dienst & Bronwell, 1984). *K. granulomatis* is pleomorphic when observed in a single plane (Dienst & Bronwell, 1984), i.e. curved or straight rods, coccoid, diplococcoid, ovoid or elliptical in shape. Mature forms are capsulated, ovoid to elliptical in shape and measure 0.5–0.7 µm in diameter and 1.0–1.5 µm in length (Rajam & Rangiah, 1954; Sehgal & Sharma, 1992; Hart, 1997). Immature forms are non-capsulated, coccoid, diplococcoid or bacillary in shape and measure 0.6–1.0 µm in length (Rajam & Rangiah, 1954; Sehgal & Sharma, 1992; Hart, 1997). Immature, non-capsulated forms may appear like closed safety pins with certain stains due to bipolar chromatin densities (Rajam & Rangiah, 1954; Sehgal & Sharma, 1992; Hart, 1997). Division is via invagination of the cell wall and cytoplasmic membrane (Anderson *et al.*, 1945; Davis & Collins, 1969; Spagnolo *et al.*, 1984; Sehgal & Sharma, 1992). Filamentous processes recognized as pili and fimbriae are present on the surface of most organisms (Dodson *et al.*, 1974; Kuberski *et al.*, 1980; Chandra & Jain, 1991). Numerous round vesicles or blebs endogenous

to the cell wall are often seen and may be attached to or detached from the cell wall (Dodson *et al.*, 1974; Kuberski *et al.*, 1980; Chandra & Jain, 1991).

Staining properties. Gram-negative. Well seen with Giemsa, Leishman, Wright's or Silver stains. Poorly visualized with haematoxylin and eosin (Richens, 1991). Periodic acid Schiff-negative (Spagnolo *et al.*, 1984; Richens, 1991).

Cultural and growth conditions. *K. granulomatis* is facultatively intracellular, residing in the cytoplasm of large mononuclear cells and occasionally within polymorphonuclear leukocytes (Dodson *et al.*, 1974; Kuberski *et al.*, 1980; Spagnolo *et al.*, 1984; Chandra & Jain, 1991). In the yolk sac of the developing chick embryo a conspicuous feature is its residence within epithelial cells (Anderson, 1943). Successful cultures have been achieved *in vivo* in the yolk sac of developing chick embryos (Anderson, 1943) and in the developing chick embryo brain (Thomison, 1951). Cultures *in vitro* have been achieved utilizing fresh yolk containing embryonic chick heart (Anderson, 1943); chick embryo amniotic fluid (Anderson *et al.*, 1945); semi-solid medium containing peptone, tryptone, dextrose, sea salt, agar and fresh yolk (Dienst, 1948); slants prepared from beef heart infusion agar and fresh yolk (Dunham & Rake, 1948); Locke-Yolk agar with a Locke solution overlay (Dulaney *et al.*, 1948); thioglycollate broth with lactalbumin hydrosylate (enzymic digest of albumin) or Phytone (enzymic digest of soya meal) added (Goldberg, 1959); fresh mononuclear cells (Kharsany *et al.*, 1996, 1997) and a human epithelial cell line (Carter *et al.*, 1997). The optimal temperature for growth of *K. granulomatis* is 37 °C (Anderson, 1943; Beveridge, 1946; Dienst & Bronwell, 1984). Two factors present within the yolk sac of developing chick embryos have been found to be essential for growth. These are a micro-aerophilic environment (Anderson, 1943; Dienst, 1948; Goldberg, 1959) and a polypeptide present in the enzymic digests of bovine albumin and soya meal (Goldberg, 1959).

Storage conditions. *K. granulomatis* will not remain viable when stored at 5 or 37 °C (Anderson, 1943). It has been reported that stock egg yolk cultures remain viable for extended periods when stored at 25 °C (Anderson, 1943) though Dienst (1948) could only maintain viability of cultures in fresh yolk medium for 8–10 d at 25 °C.

Genetic data. The mol % G + C content is unavailable due to the absence of DNA–DNA hybridization studies. *K. granulomatis* shows 99.7–99.8 % nucleotide similarity with *K. pneumoniae* and *K. rhinoscleromatis*, respectively, in the gene encoding the outer-membrane phosphate porin and 98.8–99.8 % nucleotide similarity with *K. pneumoniae* and *K. rhinoscleromatis*, respectively, in the 16S rRNA gene.

Pathogenicity and habitat. *K. granulomatis* is not pathogenic for mice, dogs, chickens, the chorion-allantoic membrane of chick embryos, rabbits, guinea pigs, *Macacus rhesus* monkeys, sheep, goats, pigs or

cows (Anderson, 1943; Anderson *et al.*, 1945; Beveridge, 1946; Dienst *et al.*, 1949). With the exception of the developing chick embryo (and other types of eggs) *K. granulomatis* is pathogenic only for humans (Dienst & Bronwell, 1984) where infection results in the chronic genital ulcerative disease known as donovanosis. *K. granulomatis* has been assumed to be sexually transmitted (Rajam & Rangiah, 1954; Sengupta, 1981; Spence, 1988; Sehgal & Sharma, 1992; Hart, 1997) though an enteric habitat has been postulated (DeMonbreun & Goodpasture, 1933; Dunham & Rake, 1948; Goldberg, 1962, 1964). Goldberg (1962) isolated and cultured *K. granulomatis* from the faeces of a patient with donovanosis and DeMonbreun & Goodpasture (1933) also claim to have achieved successful isolation (though not culture) of *K. granulomatis* from the faeces of 2/4 patients with donovanosis. It is currently unknown whether *K. granulomatis* also has a natural environmental habitat.

Type culture. Due to difficulties encountered in the storage of *K. granulomatis*, no type culture is currently available.

Acknowledgements

This study was funded by Territory Health Services, Northern Territory Government. J. S. C. is in receipt of a scholarship from the National Health and Medical Research Council of Australia.

References

- Anderson, K. (1943). The cultivation from granuloma inguinale of a microorganism having the characteristics of Donovan bodies in the yolk sac of chick embryos. *Science* **97**, 560–561.
- Anderson, K., DeMonbreun, W. A., & Goodpasture, E. W. (1945). An etiologic consideration of *Donovania granulomatis* cultivated from granuloma inguinale (three cases) in embryonic yolk. *J Exp Med* **81**, 25–40.
- Aragão, H. D. & Vianna, G. (1913). Pesquisas sobre o granuloma venereo. *Mem Inst Oswaldo Cruz* **5**, 211–238.
- Bastian, I. & Bowden, F. J. (1996). Amplification of *Klebsiella*-like sequences from biopsy samples from patients with donovanosis. *Clin Infect Dis* **23**, 1328–1330.
- Beige, J., Lokies, T., Schaberg, T., Finckh, V., Fischer, M., Mauch, H., Lode, H., Kohler, B. & Rolfs, A. (1995). Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. *J Clin Microbiol* **33**, 90–95.
- Beveridge, W. (1946). The action of antimony and some other bacteriostatic substances on *Donovania granulomatis* isolated in the chick embryo. *J Immunol* **53**, 215–223.
- Carter, J., Hutton, S., Sriprakash, K. S., Kemp, D. J., Lum, G., Savage, J. & Bowden, F. J. (1997). Culture of the causative organism of donovanosis (*Calymmatobacterium granulomatis*) in HEP-2 cells. *J Clin Microbiol* **35**, 2915–2917.
- Carter, J., Bowden, F. J., Sriprakash, K. S., Bastian, I. & Kemp, D. J. (1999). Diagnostic polymerase chain reaction for donovanosis. *Clin Infect Dis* **28**, 1168–1169.
- Chandra, M. & Jain, A. (1991). Fine structure of *Calymmatobacterium granulomatis* with particular reference to the surface structure. *Indian J Med Res* **93**, 225–231.
- Davis, C. (1970). Granuloma inguinale. A clinical, histological, and ultrastructural study. *J Am Med Assoc* **211**, 632–636.
- Davis, C. & Collins, C. (1969). An ultrastructural study of *Calymmatobacterium granulomatis*. *J Invest Dermatol* **53**, 315–320.
- DeMonbreun, W. & Goodpasture, E. (1933). Further studies on the etiology of granuloma inguinale. *Am J Trop Med* **13**, 447–463.
- Dienst, R. B. (1948). Laboratory diagnosis of granuloma inguinale and studies on the cultivation of the donovan body. *Am J Syph Gonorr Vener Dis* **32**, 301–306.
- Dienst, R. B. & Bronwell, G. H. (1984). Genus *Calymmatobacterium* Aragão and Vianna 1913, 221^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 585–587. Edited by N. R. Krieg. Baltimore, MD: Williams & Wilkins.
- Dienst, R. B., Chen, C. H. & Greenblatt, R. B. (1949). Experimental studies on the pathogenicity of *Donovania granulomatis*. *Am J Syph Gonorr Vener Dis* **33**, 152–157.
- Dodson, R., Fritz, G., Hubler, W., Jr, Rudolf, A., Knox, J. & Chu, L. (1974). Donovanosis: a morphologic study. *J Invest Dermatol* **62**, 611.
- Dulaney, A. D., Guo, K. & Packer, H. (1948). *Donovania granulomatis*: cultivation, antigen preparation, and immunological tests. *J Immunol* **59**, 335–340.
- Dunham, W. & Rake, G. (1948). Cultural and serological studies on granuloma inguinale. *Am J Syph Gonorr Vener Dis* **32**, 145–149.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Fredricks, D. N. & Relman, D. A. (1996). Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev* **9**, 18–33.
- Goldberg, J. (1959). Studies on granuloma inguinale. IV. Growth requirements of *Donovania granulomatis* and its relationship to the natural habitat of the organism. *Br J Vener Dis* **35**, 266–268.
- Goldberg, J. (1962). Studies on granuloma inguinale. V. Isolation of a bacterium resembling *Donovania granulomatis* from the faeces of a patient with granuloma inguinale. *Br J Vener Dis* **38**, 99–102.
- Goldberg, J. (1964). Studies on granuloma inguinale. VII. Some epidemiological considerations of the disease. *Brit J Vener Dis* **40**, 140–145.
- Hart, G. (1997). Donovanosis. *Clin Infect Dis* **25**, 24–30.
- Kharsany, A. B., Hoosen, A. A., Kiepiela, P., Naicker, T. & Sturm, A. W. (1996). Culture of *Calymmatobacterium granulomatis*. *Clin Infect Dis* **22**, 391.
- Kharsany, A. B., Hoosen, A. A., Kiepiela, P., Naicker, T. & Sturm, A. W. (1997). Growth and cultural characteristics of *Calymmatobacterium granulomatis*—the aetiological agent of granuloma inguinale (Donovanosis). *J Med Microbiol* **46**, 579–585.
- Kuberski, T., Papadimitriou, J. & Phillips, P. (1980). Ultrastructure of *Calymmatobacterium granulomatis* in lesions of granuloma inguinale. *J Infect Dis* **142**, 744–749.
- Levine, M. & Hoyt, R. (1947). Scleroma. Complement fixation test. *Proc Soc Exp Biol Med* **65**, 70–72.
- Maddocks, I., Anders, E. & Dennis, E. (1975). Donovanosis in Papua New Guinea. *Br J Vener Dis* **52**, 190–196.
- Mullenbach, R., Lagoda, P. & Welter, C. (1989). An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genet* **5**, 391.

- Ørskov, I. (1984). Genus V. *Klebsiella* Trevisan 1885, 105^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 461–465. Edited by N. R. Krieg. Baltimore, MD: Williams & Wilkins.
- Packer, H. & Goldberg, J. (1950). Studies of the antigenic relationship of *D. granulomatis* to members of the tribe Eschericheae. *Am J Syph Gon Vener Dis* **34**, 342–350.
- Rajam, R. V. & Rangiah, P. N. (1954). *Donovanosis*. Geneva: WHO.
- Richens, J. (1985). Donovanosis – a review. *P N G Med J* **28**, 67–74.
- Richens, J. (1991). The diagnosis and treatment of donovanosis (granuloma inguinale). *Genitourin Med* **67**, 441–452.
- Sakazaki, R., Tamura, K. & Kosako, Y. (1989). *Klebsiella ornithinolytica* sp. nov., formerly known as ornithine positive *Klebsiella oxytoca*. *Curr Microbiol* **18**, 201–206.
- Sehgal, V. & Jain, M. (1987). Tissue section donovan bodies – identification through slow giemsa (overnight) technique. *Dermatologica* **174**, 28–31.
- Sehgal, V. N. & Sharma, H. K. (1992). Donovanosis. *J Dermatol* **19**, 932–46.
- Sengupta, B. S. (1981). Vulval cancer following or co-existing with chronic granulomatous diseases of vulva. An analysis of its natural history, clinical manifestation and treatment. *Trop Doct* **11**, 110–114.
- Shaw, H. & Martin, H. (1961). Rhinoscleroma. Clinical perspective. *J Laryngol Otol* **75**, 1011–1039.
- Spagnolo, D. V., Coburn, P. R., Cream, J. J. & Azadian, B. S. (1984). Extragenital granuloma inguinale (Donovanosis) diagnosed in the United Kingdom: a clinical, histological, and electron microscopical study. *J Clin Pathol* **37**, 945–949.
- Spence, M. R. (1988). The treatment of gonorrhea, syphilis, chancroid, lymphogranuloma venereum, and granuloma inguinale. *Clin Obstet Gynecol* **31**, 453–465.
- Thomison, J. (1951). Primary isolation of *Donovania granulomatis* by chick brain inoculation, and induction of cerebral miliary granulomata. *Proc Soc Exp Biol Med* **77**, 557–578.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Welsh, R., Correa, P. & Herran, R. (1963). Light and electron microscopic observations of scleroma. *Exp Mol Pathol* **2**, 93–101.