A new phylogenetic group of *Propionibacterium acnes*

Andrew McDowell,¹ Alexandra L. Perry,² Peter A. Lambert²
and Sheila Patrick¹

Correspondence

Andrew McDowell
a.mcdowell@qub.ac.uk

¹School of Medicine and Dentistry, Queen's University, Belfast BT12 6BN, UK

²Pharmaceutical and Biological Sciences, School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK

Immunofluorescence microscopy-based identification of presumptive *Propionibacterium acnes* isolates, using the *P. acnes*-specific mAb QUBPa3, revealed five organisms with an atypical cellular morphology. Unlike the coryneform morphology seen with *P. acnes* types I and II, these isolates exhibited long slender filaments (which formed large tangled aggregates) not previously described in *P. acnes*. No reaction with mAbs that label *P. acnes* types IA (QUBPa1) and II (QUBPa2) was observed. Nucleotide sequencing of the 16S rRNA gene (1484 bp) revealed the isolates to have between 99.8 and 99.9 % identity to the 16S rRNA gene of the *P. acnes* type IA, IB and II strains NCTC 737, KPA171202 and NCTC 10390, respectively. Analysis of the *recA* housekeeping gene (1047 bp) did reveal, however, a greater number of conserved nucleotide polymorphisms between the sequences from these isolates and those from NCTC 737 (98.9 % identity), KPA171202 (98.9 % identity) and NCTC 10390 (99.1 % identity). Phylogenetic investigations demonstrated that the isolates belong to a novel *recA* cluster or lineage distinct from *P. acnes* types I and II. We now propose this new grouping as *P. acnes* type III. The prevalence and clinical importance of this novel *recA* lineage amongst isolates of *P. acnes* remains to be determined.

Received 2 July 2007

Accepted 12 October 2007

INTRODUCTION

Propionibacterium acnes belongs to the 'high GC' group of Gram-positive bacteria and is found predominately in the sebaceous gland-rich areas of the skin in adults (McGinley *et al.*, 1978). Although traditionally considered a relatively non-pathogenic member of the resident human microbiota, an increasing number of studies have implicated *P. acnes* as the agent responsible for various clinical conditions and infections. In addition to its well described role in inflammatory acne (Eady & Ingham, 1994), *P. acnes* is emerging as an important pathogen in relation to medical implant-related infections, such as those associated with central nervous system shunts (Brook & Frazier, 1991), silicone implants (Ahn *et al.*, 1996), and prosthetic heart valves and hip joints (Delahaye *et al.*, 2005; Tunney *et al.*, 1998, 1999). Furthermore, *P. acnes* is responsible for endophthalmitis, ocular and periocular infections (Aldave

et al., 1999; Clark *et al.*, 1999; Horgan *et al.*, 1999), as well as periodontal and dental infections (Debelian *et al.*, 1992; LeGoff *et al.*, 1997), and has been linked to synovitis–acne–pustulosis–hyperostosis–osteitis (SAPHO) syndrome (Kotilainen *et al.*, 1996), sarcoidosis (Eishi *et al.*, 2002) and prostate cancer (Cohen *et al.*, 2005). The identification of a wide range of putative virulence determinants within the recently published genome sequence of the *P. acnes* strain KPA17202 has further brought the pathogenic potential of this organism into focus (Bruggemann *et al.*, 2004).

Two distinct phenotypes of *P. acnes* (types I and II), which can be distinguished by serological agglutination tests and cell-wall sugar analysis, have been known for over 30 years (Johnson & Cummins, 1972). Additional studies have shown that these biovars display differences in the fermentation of sugar and sugar alcohols (Higaki *et al.*, 2000; Kishishita *et al.*, 1979), as well as their susceptibility to bacteriophage infection (Webster & Cummins, 1978). Sequence analysis of the *P. acnes recA* gene has revealed that types I and II correspond to phylogenetically distinct clusters or lineages (McDowell *et al.*, 2005). These two clusters are, however, almost identical based on 16S rRNA sequencing. Analysis of the *recA* gene has also identified a subcluster of strains within *P. acnes* type I that have been

Abbreviations: IFM, immunofluorescence microscopy; NCTC, National Collection of Type Cultures; ROH, Royal Orthopaedic Hospital.

The GenBank/EMBL/DDBJ accession numbers for the *recA* and 16S RNA sequences of *Propionibacterium acnes* reported in this paper are DQ672246–DQ672256 and DQ672257–DQ672261, respectively.

A figure of *recA* sequence alignments is available as supplementary data with the online version of this paper.

designated type IB (McDowell *et al.*, 2005; Valanne *et al.*, 2005). These organisms do not react with the previously described mAb QUBPa1, specific for all other type I organisms (known as type IA) (McDowell *et al.*, 2005; Valanne *et al.*, 2005). However, variable labelling with the type II mAb QUBPa2, ranging from no reaction to a weak reaction, has been observed. In the latter case a significantly reduced fluorescence intensity and a reduction in the proportion of the bacterial population labelled is seen (McDowell *et al.*, 2005). Type IB organisms also display differences from type IA strains in the production of putative Christie–Atkins–Munch–Peterson (CAMP) factor proteins (Valanne *et al.*, 2005).

This paper describes the identification of a novel lineage of *P. acnes* based on *recA* sequence analysis, which we now formally propose as *P. acnes* type III. The isolates also display differences from *P. acnes* types I and II in their cell surface antigens and cellular morphology.

METHODS

Bacterial strains. The *P. acnes* reference strains NCTC 737 (type IA, ATCC6919) and NCTC 10390 (type II, ATCC12930) were obtained from the National Collection of Type Cultures (NCTC) (London, UK). A total of four isolates of *P. acnes* type III were recovered from spine intervertebral disc material removed during microdissectomy procedures (for relief of severe sciatica), while one isolate was recovered from a prosthetic hip joint removed during revision arthroplasty. All surgeries were performed at the Royal Orthopaedic Hospital (ROH), Birmingham, England. A further 95 isolates of *P. acnes* (types I & II) were also included in our study for comparative purposes. A total of 15 of these isolates were recovered from failed prosthetic hip joints, as well as associated tissue, removed during revision arthroplasties; while a further 34 isolates were isolated from spine intervertebral disc material removed during microdissectomy procedures at ROH. A total of 13 isolates from routine blood cultures, 18 from acne lesions and 15 from normal skin were obtained from the Queen Elizabeth Hospital, Birmingham, England. Isolates of *Propionibacterium granulosum* and *Propionibacterium avidum* recovered from patients with acne lesions were a kind gift from Professor Keith Holland. Ethical approval was obtained from the South Birmingham Local Research Ethics Committee and informed patient consent was obtained in all cases.

Bacterial culture. Strains were grown on anaerobic blood agar (ABA) (CM0972; Oxoid). Cultures were incubated at 37 °C in an anaerobic cabinet (MACS MG 1000; Don Whitley Scientific), in an atmosphere of 80 % N₂, 10 % CO₂ and 10 % H₂. Isolates were examined using the API ID 32A and API 20A biochemical identification systems (bioMérieux) in accordance with the manufacturer's instructions. Fermentation analyses with the substrates sorbitol, ribose and erythritol were carried out as previously described (McDowell *et al.*, 2005). Putative virulence factors, namely haemolytic, proteinase, lipase, lecithinase, DNase, elastase and hyaluronidase activities, were detected as reported by Balke & Weiss (1984) and Spare *et al.* (2003).

Immunofluorescence microscopy (IFM). IFM was conducted on multiwell slides as described previously (McDowell *et al.*, 2005). Strains were examined with the mAbs QUBPa1 and QUBPa2, which label *P. acnes* type IA and II, respectively, as well as mAb QUBPa3, which reacts with all *P. acnes* strains examined to date (Tunney *et al.*,

1999). Slides were read using a Leitz Dialux 20 fluorescence microscope. Images were captured and bacterial cell lengths determined using LUCIA G software following the manufacturer's instructions (Laboratory Imaging).

Nucleotide sequence analysis. Nucleotide sequence analysis was performed on 16S rRNA and *recA* genes. The 16S rRNA gene (1484 bp) was amplified using the universal primers UFPL and URPL (LiPuma *et al.*, 1999), while *recA* was amplified with the primers PAR-1 and PAR-2, which are directed to downstream and upstream flanking sequences of the *recA* ORF, respectively, and generate a 1201 bp amplicon (McDowell *et al.*, 2005). PCR products were verified by electrophoresis on 1 % (w/v) agarose gels and duplicate samples for each gene pooled before purification with a QIAquick PCR purification kit (Qiagen) (McDowell *et al.*, 2005). Sequencing reactions were performed using ABI PRISM ready reaction terminator cycle sequencing kits (version 1.1) (Perkin-Elmer Applied Biosystems) according to the manufacturer's instructions and the samples analysed on an ABI PRISM 3100 genetic analyser capillary electrophoresis system (Perkin-Elmer Applied Biosystems). Raw sequences from both DNA strands were obtained by using the appropriate forward and reverse primers. Internal sequencing primers were also used to facilitate determination of the larger 16S rRNA gene sequence. Initial sequences were screened using the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov) to confirm gene identity.

Phylogenetic analysis. Multiple nucleotide sequence alignments (with no gaps) of the *recA* gene (1047 bp) from all five atypical isolates, as well as complete *recA* sequences for previously published strains representative of types I and II (McDowell *et al.*, 2005), were constructed using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic analyses were conducted using the genetic distance-based neighbour-joining algorithms within MEGA version 3.1 (<http://www.megasoftware.net/>) and phylogenetic trees constructed using the Jukes–Cantor matrix model. The sequence input order was randomized and bootstrapping resampling statistics were performed using 100 datasets for each analysis.

Nucleotide sequence accession numbers. Nucleotide sequences for 16S rRNA and *recA* gene sequences generated during this study were submitted to GenBank and the accession numbers DQ672257–DQ672261 and DQ672246–DQ672256 generated, respectively.

RESULTS AND DISCUSSION

IFM

During routine IFM-based identification of clinical isolates presumptively identified as *P. acnes* by biochemical analysis, five organisms (designated Asn10–Asn14) with an atypical morphology were identified after labelling with the mAb QUBPa3, which reacts with a carbohydrate or glycolipid-containing antigen on the surface of all *P. acnes* cells (Tunney *et al.*, 1999). Four of these isolates were recovered from spine intervertebral disc material (excised disc protrusion) removed during microdissectomy procedures for severe sciatica, while one was from a prosthetic hip arthroplasty. Compared to the classical coryneform morphology normally seen with types I and II (i.e. clubs, 'tadpole' forms and short bifid forms), these isolates consisted of individual cells of variable length and long slender filaments that formed very large tangled aggregates

(Figs 1 and 2). This was also observed upon Gram staining of the cells (not illustrated). No labelling with the mAbs QUBPa1 and QUBPa2, which react with a proteinaceous and carbohydrate/glycolipid-containing antigen on types IA and II (McDowell *et al.*, 2005), respectively, was detected for any of the isolates. This provides evidence that the cell surface antigens recognized by these mAbs either are not present, or are structurally distinct to those found on types I and II giving rise to a different set of epitopes.

Analysis of cells from one of these isolates (Asn12), captured from three different fields of view of an IFM image using a $\times 100$ objective, revealed individual cells and filaments that varied in length from 1.20 to 21.8 μm , with widths ranging from 0.71 to 0.96 μm . However, much longer filaments, which could be difficult to identify due to a greater tendency to form or to be associated with large aggregates, may also be present. For comparison, analysis of individual cells of NCTC 737 (type IA) and NCTC 10390 (type II) in a similar manner revealed lengths that ranged from 0.84 to 2.56 μm and 0.89 to 2.80 μm , respectively, with widths ranging from 0.63 to 0.88 μm and 0.66 to 0.86 μm , respectively. While filaments have not been described before in *P. acnes*, slender or fine branching filaments (5–20 μm or greater in length) are a characteristic feature of *Propionibacterium propionicum* (previously

Fig. 2. Micrograph of a large filamentous aggregate from the *P. acnes* type III isolate Asn12 (after growth on an ABA plate). Filaments were immunolabelled with the mouse IgG mAb QUBPa3 and a FITC-conjugated goat anti-mouse IgG antibody (magnification $\times 100$).

Arachnia propionica), along with short irregular rods of variable length that are commonly arranged in pairs (Holt *et al.*, 1994). Unlike the atypical *P. acnes* isolates described in this study, however, *P. propionicum* does not show any reaction with the mAb QUBPa3 (A. McDowell & S. Patrick, unpublished data). Filaments that are associated with branching and hyphal formation are also characteristic of other members of the *Actinomycetales*, such as the *Nocardioles*, and the less closely related *Actinomyces* and *Rhodococcus* (Holt *et al.*, 1994). It is interesting to note that in addition to resembling *Actinomyces israelii* morphologically, *P. propionicum* can also cause actinomycosis, a chronic, subacute suppurating granulomatous infection (Hall, 2006). Whether these *P. acnes* strains also share any similarities with *P. propionicum* and *Actinomyces* spp. in terms of pathogenicity remains to be determined. Their ability to form filaments may impact on the potential for colonization, particularly in relation to tissue penetration and biofilm formation. It is possible that type III isolates have remained unidentified as *P. acnes* due to their atypical morphology, as would be observed upon routine Gram staining.

Nucleotide sequence and phylogenetic analysis

Systematic analysis of all five atypical *P. acnes* isolates was initially conducted by direct nucleotide sequencing of a 1484 bp fragment of the 16S rRNA gene amplified with the universal 16S rRNA-based primers UFPL and URPL. The GenBank accession numbers for these 16S sequences are listed in Table 1. Upon CLUSTAL W analysis, sequences obtained for all the isolates had between 99.8 to 99.9% identity to the previously published sequences for NCTC 737 (type IA, GenBank accession no. AB042288) KPA171202 (type IB, GenBank accession no. NC_006085) and NCTC 10390 (type II, GenBank accession no. AY642044), thus confirming the very close relationship between these organisms and types I and II. In contrast, when a 1473 bp sequence stretch of the 16S rRNA gene

Fig. 1. Micrographs of *P. acnes* (after growth on ABA plates) immunolabelled with the mouse IgG mAb QUBPa3 and a FITC-conjugated goat anti-mouse IgG antibody (magnification $\times 100$): (a) Asn10 (type III), (b) Asn12 (type III), (c) NCTC 737 (type IA), (d) NCTC 10390 (type II).

Table 1. Nucleotide sequence analysis of *P. acnes* isolates with atypical morphology

Isolate	GenBank 16S rRNA accession no.	GenBank <i>recA</i> accession no.
Asn10	DQ672257	DQ672252
Asn11	DQ672258	DQ672253
Asn12	DQ672259	DQ672254
Asn13	DQ672260	DQ672255
Asn14	DQ672261	DQ672256

from all five isolates was aligned and compared with the sequence from *P. propionicum* strain DSM 43307 (GenBank accession no. AJ003058) much greater differences were observed (95.8–95.9 % identity). Although sequencing of the 16S rRNA gene is still considered the ‘gold-standard’ for investigating the phylogenetic relationship between bacterial organisms, potential problems associated with its ability to resolve the relationships between closely related species, due to an extremely low rate of neutral mutation, have been recognized (Vandamme *et al.*, 1996). In contrast, protein-encoding genes with housekeeping functions, such as *recA*, often provide a better foundation for bacterial systematics and differentiation of closely related organisms (Eisen, 1995; Mahenthiralingam *et al.*, 2000). This is due to a higher neutral mutation rate within such genes, which is a consequence of the redundancy of the genetic code resulting in synonymous substitutions at the third codon position that have no effect on the function of the resulting protein. Since we previously found that analysis of the *recA* housekeeping gene provided much greater insight into the phylogenetic relationship between *P. acnes* types I and II compared to 16S rRNA (McDowell *et al.*, 2005), it was a natural and pertinent step to further investigate potential phylogenetic differences between these five atypical isolates of *P. acnes* and types I and II based on this locus.

A 1201 bp fragment containing the *recA* gene was successfully amplified from all five atypical isolates, using the previously described primers PAR-1 and PAR-2 (McDowell *et al.*, 2005), and sequenced. The GenBank accession numbers for these *recA* sequences are listed in Table 1. Other closely related *Propionibacterium* species, such as *P. granulosum*, do not show any reaction with this particular primer pair (A. McDowell & S. Patrick, unpublished data). Upon CLUSTAL W analysis, the *recA* sequences (1047 bp) for these isolates showed distinct differences from those previously published for NCTC 737 (GenBank accession no. AY642055, 98.9 % identity), KPA171202 (GenBank accession no. NC_006085, 98.9 % identity) and NCTC 10390 (GenBank accession no. AY642061, 99.1 % identity). Strains of *P. acnes* type I (IA & IB) and II differ in 10 highly conserved regions within the *recA* gene (99 % identity). For our group of atypical isolates, the *recA* gene sequences were found to contain a combination of four type I-specific polymorphisms (common to IA and IB), six type II-specific polymorphisms and

five polymorphisms unique to the group (the sequence alignments are shown in Supplementary Fig. S1 available with the online journal). To investigate the phylogenetic relationship between these five isolates and types I and II, a *recA* phylogenetic tree was constructed based on the nucleotide sequences (Fig. 3). Previously published *recA* sequences for types IA, IB and II were included in the analysis for comparison (McDowell *et al.*, 2005). Strains of *P. acnes* types I, II and this novel grouping formed highly distinct branches within the tree supported by bootstrap values of 98 % or 100 %. This demonstrates that the atypical isolates identified by IFM represent a new or novel *recA* phylogenetic cluster or lineage, which we propose as *P. acnes* type III. To facilitate further studies of the type III grouping the isolate Asn12 (recovered from intervertebral disc material) will be deposited in the NCTC bacterial cell bank as a representative of this novel phylogenetic cluster.

These results again highlight the potential limitations to the use of the 16S rRNA locus alone in understanding bacterial phylogeny. This may be especially important for studies that attempt to identify novel bacterial groupings or phylotypes within microbial communities, such as those present on the skin, using culture-independent 16S rRNA-based methods (Dekio *et al.*, 2005). For such investigations the use of protein-encoding genes with housekeeping functions may provide more valuable information for

Fig. 3. Unrooted phylogenetic tree of *P. acnes* based on the complete *recA* gene sequence, illustrating the three *recA* lineages of the organism. Multiple sequence alignments were performed on the *recA* gene sequences from type III isolates and published sequences representative of type IA, IB and II isolates (McDowell *et al.*, 2005) (with no gaps in the aligned sequence). Bootstrapping resampling statistics was performed using 100 datasets, with bootstrap values shown on the arms of the tree. The type status for the different strains analysed is also shown.

Table 2. Production of virulence factors by *P. acnes* types I, II and novel group III

Phylogenetic group	No. of isolates producing each putative virulence factor (percentage total of each type)*					
	α -Haemolysis	β -Haemolysis	Lipase	Lecithinase	Proteinase	Hyaluronidase
I (<i>n</i> =75)	2 (3)	50 (67)	61 (81)	19 (25)	30 (40)	69 (92)
II (<i>n</i> =20)	4 (20)	0 (0)	5 (25)	13 (65)	7 (35)	19 (95)
III (<i>n</i> =5)	0 (0)	0 (0)	5 (100)	0 (0)	2 (40)	2 (40)
Total (<i>n</i> =100)	6 (6)	50 (50)	71 (71)	32 (32)	39 (39)	90 (90)

*All isolates negative for DNase and elastase activity.

certain taxa (Palys *et al.*, 1997). Indeed, we have also constructed an accurate phylogeny of *P. acnes* (congruent with *recA* phylogeny) based on sequence analysis of putative virulence genes (McDowell *et al.*, 2005; Valanne *et al.*, 2005). This demonstrates that where rates of sequence divergence are appropriate, non-housekeeping gene sequences can also provide data that may be useful for bacterial systematics. Although *P. acnes* isolates can be assigned to the type III phylogenetic grouping based on analysis of their cellular morphology (morphovars), we would recommend sequencing of the *recA* gene for unambiguous classification. On the basis of previous DNA–DNA hybridization studies (Johnson & Cummins, 1972), types I and II would appear to represent distinct sequence clusters within *P. acnes* rather than novel species, at least based on current definitions of a bacterial species (Vandamme *et al.*, 1996; Cohan, 2002). Similar experiments will now have to be carried out with isolates from the type III grouping to confirm if this is also the case for these organisms.

Biochemical analysis

Unequivocal identification of the type III isolates as *P. acnes* (99.9% identity) was observed on the basis of analysis with the API ID32A and API 20A biochemical galleries. All type III isolates were positive for indole and nitrate reduction characteristic of *P. acnes*, but not *P. granulosum* or *P. avidum* (Holt *et al.*, 1994). The isolates were negative for sorbitol and erythritol fermentation, but could ferment ribose (biotype 4), as well as glucose and glycerol. The production of acid from mannose was variable between the isolates. In keeping with other *P. acnes* strains, the type III isolates did not produce acid from lactose, salicin, xylose, maltose, arabinose, cellobiose, melezitose, raffinose or rhamnose, and were negative for urease and β -glucosidase activity. All type III organisms were positive for catalase activity, but arginine dihydrolase activity was variable.

In addition to studies focused on understanding the metabolic profile of type III organisms, we also investigated possible differences in the production of various virulence factors between all type III isolates and 95 *P. acnes* isolates representing types I (*n*=75) and II (*n*=20) (Table 2). These

isolates, which were recovered from different sources at the ROH and Queen Elizabeth Hospital, had been routinely identified to the level of type I or II on the basis of sugar fermentation profiles (Higaki *et al.*, 2000), random amplification of polymorphic DNA fingerprints (Perry *et al.*, 2003) and, for some samples, IFM (with QUBPa1 and QUBPa2) (McDowell *et al.*, 2005). All type III isolates were positive for lipase activity, but variable in their production of proteinases and hyaluronidase. They were, however, negative for lecithinase activity, as well as α - and β -haemolytic activities. Type I isolates were found to be variable for α - and β -haemolysis, while type II isolates were negative for β -haemolysis, but did display variable α -haemolytic activity. The production of lipase, lecithinase, proteinases and hyaluronidase activities was variable for type I and II isolates (Table 2). No DNase or elastase activity was detected in any of the *P. acnes* isolates examined. PCR analysis of type III isolates revealed the presence of the co-haemolysin or CAMP factor gene family, as well as the *tly* gene encoding a putative haemolysin/cytotoxin (not illustrated), which have already been described in types I and II (Valanne *et al.*, 2005; McDowell *et al.*, 2005). Further studies with a greater range of isolated type III strains will be required to confirm all these observations. Also, continued investigation of the virulence profile of the various *P. acnes* types is warranted, especially as distinct phylogenetic groups of an organism can display differences in their pathogenic potential (Ishii *et al.*, 2007).

In conclusion, we have identified a novel phylogenetic grouping or lineage of *P. acnes* that we propose as type III. The identification of a third phylogenetic cluster in *P. acnes* further challenges our understanding of this organism, highlights potential caveats in the use of only one isolate type in laboratory studies of *P. acnes* virulence, and raises the possibility that other phylogenetic groups of the organism may exist.

ACKNOWLEDGEMENTS

We acknowledge Mr Gisli Einarsson and Dr Isaac Chen for technical assistance. A.M. was funded by a programme grant from the Northern Ireland Health and Personal Social Services Research and Development Office (NIPHS R and D Office). A.P. was funded by the University Hospital, Birmingham NHS Trust.

REFERENCES

- Ahn, C. Y., Ko, C. Y., Wagar, E. A., Wong, R. S. & Shaw, W. W. (1996). Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg* **98**, 1225–1229.
- Aldave, A. J., Stein, J. D., Deramo, V. A., Shah, G. K., Fischer, D. H. & Maguire, J. I. (1999). Treatment strategies for postoperative *Propionibacterium acnes* endophthalmitis. *Ophthalmology* **106**, 2395–2401.
- Balke, E. & Weiss, R. (1984). A simple method for rapid detection of bacterial hyaluronidase in K hyaluronate-containing gel. *Zentralbl Bakteriol Mikrobiol Hyg [A]* **257**, 317–322 (in German).
- Brook, I. & Frazier, E. H. (1991). Infections caused by *Propionibacterium* species. *Rev Infect Dis* **13**, 819–822.
- Bruggemann, H., Henne, A., Hoster, F., Liesegang, H., Wiezer, A., Strittmatter, A., Hujer, S., Durre, P. & Gottschalk, G. (2004). The complete genome sequence of *Propionibacterium acnes*, a commensal of human skin. *Science* **305**, 671–673.
- Clark, W. L., Kaiser, P. K., Flynn, H. W., Belfort, A., Miller, D. & Meisler, D. M. (1999). Treatment strategies and visual acuity outcomes in chronic postoperative *Propionibacterium acnes* endophthalmitis. *Ophthalmology* **106**, 1665–1670.
- Cohan, F. M. (2002). What are bacterial species? *Annu Rev Microbiol* **56**, 457–487.
- Cohen, R. J., Shannon, B. A., McNeal, J. E., Shannon, T. & Garrett, K. L. (2005). *Propionibacterium acnes* associated with inflammation in radical prostatectomy specimens: a possible link to cancer evolution? *J Urol* **173**, 1969–1974.
- Debelian, G. J., Olsen, I. & Tronstad, L. (1992). Profiling of *Propionibacterium acnes* recovered from root canal and blood during and after endodontic treatment. *Endod Dent Traumatol* **8**, 248–254.
- Dekio, I., Hayashi, H., Sakamoto, M., Kitahara, M., Nishikawa, T., Suematsu, M. & Benno, Y. (2005). Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *J Med Microbiol* **54**, 1231–1238.
- Delahaye, F., Fol, S., Celard, M., Vandenesch, F., Beaune, J., Bozio, A. & de Gevigney, G. (2005). *Propionibacterium acnes* infective endocarditis. Study of 11 cases and review of literature. *Arch Mal Coeur Vaiss* **98**, 1212–1218 (in French).
- Eady, E. A. & Ingham, E. (1994). *Propionibacterium acnes* – friend or foe? *Rev Med Microbiol* **5**, 163–173.
- Eisen, J. A. (1995). The RecA protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of RecAs and 16S rRNAs from the same species. *J Mol Evol* **41**, 1105–1123.
- Eishi, Y., Suga, M., Ishige, I., Kobayashi, D., Yamada, T., Takemura, T., Takizawa, T., Koike, M., Kudoh, S. & other authors (2002). Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. *J Clin Microbiol* **40**, 198–204.
- Hall, V. (2006). Anaerobic actinomycetes and related organisms. In *Principles and Practice of Clinical Bacteriology*, pp. 575–586. Edited by S. H. Gillespie & P. M. Hawkey. Chichester: Wiley.
- Higaki, S., Kitagawa, T., Kagoura, M., Morohashi, M. & Yamagishi, T. (2000). Correlation between *Propionibacterium acnes* biotypes, lipase activity and rash degree in acne patients. *J Dermatol* **27**, 519–522.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors) (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore, MD: Williams and Wilkins.
- Horgan, S. E., Matheson, M. M., McLoughlin-Borlace, L. & Dart, J. K. (1999). Use of a low nutrient culture medium for the identification of bacteria causing severe ocular infection. *J Med Microbiol* **48**, 701–703.
- Ishii, S., Meyer, K. P. & Sadowsky, M. J. (2007). Relationship between phylogenetic groups, genotypic clusters and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. *Appl Environ Microbiol* **73**, 5703–5710.
- Johnson, J. L. & Cummins, C. S. (1972). Cell wall composition and deoxyribonucleic acid similarities among anaerobic coryneforms, classical propionibacteria, and strains of *Arachnia propionica*. *J Bacteriol* **109**, 1047–1066.
- Kishishita, M., Ushijima, T., Ozaki, Y. & Ito, Y. (1979). Biotyping of *Propionibacterium acnes* isolated from normal human facial skin. *Appl Environ Microbiol* **38**, 585–589.
- Kotilainen, P., Merilahti-Palo, R., Lehtonen, O. P., Manner, I., Helander, I., Mottonen, T. & Rintala, E. (1996). *Propionibacterium acnes* isolated from sternal osteitis in a patient with SAPHO syndrome. *J Rheumatol* **23**, 1302–1304.
- Le Goff, A., Bunetel, L., Mouton, C. & Bonnaure-Mallet, M. (1997). Evaluation of root canal bacteria and their antimicrobial susceptibility in teeth with necrotic pulp. *Oral Microbiol Immunol* **12**, 318–322.
- LiPuma, J. J., Dulaney, B. J., McMenamin, J. D., Whitby, P. W., Stull, T. L., Coenye, T. & Vandamme, P. (1999). Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. *J Clin Microbiol* **37**, 3167–3170.
- Mahenthalingam, E., Bischof, J., Byrne, S. K., Radomski, C., Davies, J. E., Av-Gay, Y. & Vandamme, P. (2000). DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* **38**, 3165–3173.
- McDowell, A., Valanne, S., Ramage, G., Tunney, M. M., Glenn, J. V., McLorinan, G. C., Bhatia, A., Maisonneuve, J. F., Lodes, M. & other authors (2005). *Propionibacterium acnes* types I and II represent phylogenetically distinct groups. *J Clin Microbiol* **43**, 326–334.
- McGinley, K. J., Webster, G. F. & Leyden, J. J. (1978). Regional variations of cutaneous propionibacteria. *Appl Environ Microbiol* **35**, 62–66.
- Palys, T., Nakamura, L. K. & Cohan, F. M. (1997). Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int J Syst Bacteriol* **47**, 1145–1156.
- Perry, A. L., Worthington, T., Hilton, A. C., Lambert, P. A., Stirling, A. J. & Elliott, T. S. J. (2003). Analysis of clinical isolates of *Propionibacterium acnes* by optimised RAPD. *FEMS Microbiol Lett* **228**, 51–55.
- Spare, M. K., Tebbs, S. E., Lang, S., Lambert, P. A., Worthington, T., Lipkin, G. W. & Elliott, T. S. J. (2003). Genotypic and phenotypic properties of coagulase negative staphylococci causing dialysis catheter related sepsis. *J Hosp Infect* **54**, 272–278.
- 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.
- Tunney, M. M., Patrick, S., Gorman, S. P., Nixon, J. R., Anderson, N., Davis, R. I., Hanna, D. & Ramage, G. (1998). Improved detection of infection in hip replacements. *J Bone Joint Surg Br* **80**, 568–572.
- Tunney, M. M., Patrick, S., Curran, M. D., Ramage, G., Hanna, D., Nixon, J. R., Gorman, S. P., Davis, R. I. & Anderson, N. (1999). Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *J Clin Microbiol* **37**, 3281–3290.
- Valanne, S., McDowell, A., Ramage, G., Tunney, M. M., Einarsson, G. G., O'Hagan, S., Wisdom, G. B., Fairley, D., Bhatia, A. & other authors (2005). CAMP factor homologues in *Propionibacterium acnes*: a new protein family differentially expressed by types I and II. *Microbiology* **151**, 1369–1379.

Vandamme, P., Pot, B., Gillis, M., Devos, P., Kersters, K. & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* **60**, 407–438.

Webster, G. F. & Cummins, C. S. (1978). Use of bacteriophage typing to distinguish *Propionibacterium acnes* types I and II. *J Clin Microbiol* **7**, 84–90.