Analysis of clinical isolates of *Propionibacterium acnes* by optimised RAPD

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**Abstract**

Random amplification of polymorphic DNA (RAPD) was evaluated as a genotypic method for typing clinical strains of *Propionibacterium acnes*. RAPD can suffer from problems of reproducibility if parameters are not standardised. In this study the reaction conditions were optimised by adjusting template DNA concentration and buffer constituents. All isolates were typeable using the optimised RAPD protocol which was found to be highly discriminatory (Simpson’s diversity index, 0.98) and reproducible. Typing of *P. acnes* by optimised RAPD is an invaluable tool for the epidemiological investigation of *P. acnes* for which no other widely accepted method currently exists.

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**Keywords:** Propionibacterium acnes; Random amplification of polymorphic DNA; Molecular typing

1. **Introduction**

*Propionibacterium acnes* is a Gram-positive, non-spore-forming, anaerobic bacillus, which is commonly present as part of the normal flora of the skin, oral cavity, large intestine and the external ear [1]. Historically *P. acnes* is considered to be of low virulence; however, in recent years it has been found as the aetiologic agent in various pathologies. It is most notably implicated in the condition acne vulgaris [2] but is also associated with endophthalmitis [3], endocarditis [4], osteomyelitis [5], sarcoidosis [6] and prosthetic hip infections [7]. Recently *P. acnes* has been isolated from intervertebral disc material from patients with severe sciatica [8]. It is proposed that the microorganism might be causing a chronic low-grade infection in the lower vertebral discs of patients with severe sciatica [8].

Molecular typing of *P. acnes* and other cutaneous propionibacteria has not been widely applied in the clinical situation. Dairy propionibacteria have been analysed previously by random amplification of polymorphic DNA (RAPD) and restriction endonuclease analysis [9], and pulsed field gel electrophoresis has been used to compare strains of *P. acnes* isolated from cases of endophthalmitis [10]. However, in our experience utilisation of these methods has provided data which are less than satisfactory, time-consuming and expensive to implement. Progress in molecular typing of *P. acnes* has been hindered by the lack of an effective cell lysis technique; however, this has now been overcome to some extent by the use of penicillin in the lysis method [11].

RAPD is a rapid method which uses a single short primer allowing for the detection of DNA sequence polymorphisms. We investigated the potential of RAPD to genotype strains of *P. acnes* isolated from clinical samples. Amplification-based DNA fingerprinting methods can be sensitive to changes in reaction conditions resulting in changes in profiles and therefore careful optimisation of typing protocols is required [12]. In this study we describe an optimised RAPD protocol for the epidemiological typing of *P. acnes* from clinical sources. Such a method will...
provide invaluable epidemiological data for *P. acnes* which is currently lacking.

2. Materials and methods

2.1. Bacterial strains

Clinical strains of *P. acnes* (*n* = 46) were obtained from the University Hospital Birmingham NHS Trust and identified by analytical profile index (bioMérieux). *P. acnes* NCTC 737 and 10390 were included as reference strains and laboratory strains of *Propionibacterium granulosum* (*n* = 1) and *Propionibacterium avidum* (*n* = 3) as related (outlying) species.

2.2. DNA extraction

Strains were grown in 20 ml brain heart infusion (Oxoid, UK) in universal bottles and incubated at 37°C without shaking for 72 h. The cultures were then supplemented with 20 μl penicillin G (20 mg ml⁻¹) and incubated for a further 3 h. Genomic DNA was prepared by sodium dodecyl sulfate (SDS) lysis and ethanol precipitation as follows: cells were harvested from 6 ml of culture (13000 × g, 5 min) and the pellet was resuspended in 270 μl Tris–EDTA (10 mM Tris, 1 mM EDTA, pH 8.0). The cells were heated at 75°C for 10 min to inactivate DNases and lysed by addition of 30 μl SDS (100 mg ml⁻¹). The lystate was treated with 3 μl proteinase K (10 mg ml⁻¹) and the suspension incubated at 65°C for 3 h. The suspension was diluted to 600 μl with sterile distilled water and an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added. After brief vortexing and centrifugation (13000 × g, 5 min) and the upper aqueous phase containing the DNA was recovered and precipitated by addition of 0.1 vol 3 M sodium acetate pH 5.2 and 2 vol ice-cold ethanol. The suspension was incubated at −20°C for 20 min and the DNA pelleted at 13000 × g × 20 min. Following a 70% (v/v) ethanol wash the DNA pellet was dried at 40°C for 10 min. The pellet was redissolved in 30 μl polymerase chain reaction (PCR) grade water and allowed to rehydrate overnight at 4°C. DNA samples were checked for purity and quantified by spectrophotometric measurement (optical density ratio 260/280 nm). All preparations were stored at −20°C until required.

2.3. RAPD optimisation

The optimum buffer composition for PCR reactions was determined using the Opti-Prime® buffer matrix (Stratagene, USA) consisting of 12 buffers varying in MgCl₂ and KCl concentrations and pH (Table 1). The buffer supporting amplification of profiles with suitable discrimination was selected for further testing. A range of DNA concentrations (3–40 ng ml⁻¹) were subjected to RAPD analysis to establish the optimum concentration of template.

2.4. Optimised RAPD reaction

PCR cycling conditions were performed as described by Hilton et al. [13] using the selected buffer and DNA concentration. PCR was carried out in a 25 μl reaction containing 1× PCR buffer, 200 μM dNTPs (Promega, UK), 100 pmol primer 1254 (5'-CCGCAGCACAAG-3', MWG biotech, Ebersberg, Germany), 1.25 U *Taq* polymerase (Promega, UK) and 2 μl of DNA template. The amplification procedure comprised of one cycle for 4.5 min at 94°C followed by five cycles of 30 s at 94°C, 2 min at 20°C and 2 min at 72°C, and 35 cycles of 30 s at 94°C, 1 min at 32°C and 2 min at 72°C (PTC-100 Peltier Thermal Cycler, MJ Research, Inc., USA). The amplification was concluded with a final extension step of 5 min at 72°C and the reactions stored at −20°C. Amplification products were separated by electrophoresis in 2% agarose and visualised by ethidium bromide staining. RAPD fingerprints were analysed using GelCompar (Applied Maths, Belgium) with the band matching coefficient of Dice and a dendrogram generated using UPGMA clustering. To confirm intra- and inter-reproducibility using the selected optimum quantity of DNA and PCR buffer, RAPD was performed on DNA samples in duplicate and on samples of DNA template prepared from separate cell cultures. The discriminatory power of the typing method was calculated using Simpson’s diversity index (DI) [14].

3. Results and discussion

3.1. RAPD optimisation – buffer composition

Profiles from PCR reactions using 12 buffers varying in pH and MgCl₂ and KCl concentrations are shown in Fig.

| Table 1
| Opti-Prime® buffer matrix [19] |
|-----------------------------|
| **Tris–HCl (10 mmol l⁻¹)** | **MgCl₂ (mmol l⁻¹)** | **KCl (25 mmol l⁻¹)** | **KCl (75 mmol l⁻¹)** |
| pH 8.3 | 1.5 | buffer 1 | buffer 2 |
| pH 8.3 | 3.5 | buffer 3 | buffer 4 |
| pH 8.8 | 1.5 | buffer 5 | buffer 6 |
| pH 8.8 | 3.5 | buffer 7 | buffer 8 |
| pH 9.2 | 1.5 | buffer 9 | buffer 10 |
| pH 9.2 | 3.5 | buffer 11 | buffer 12 |
1. At each pH, a high concentration of MgCl₂ (3.5 mM) with a low concentration of KCl (25 mM) did not support product amplification (lanes 3, 7 and 11). However, a decrease in MgCl₂ concentration to 1.5 mM with a low or high concentration of KCl supported product formation but gave smeared profiles with low discrimination (lanes 2, 4, 6, 8, 10 and 12). Increasing the pH appeared to have little effect on the profiles obtained relative to the effect of changing the concentrations of KCl and MgCl₂. Similar effects of buffer composition upon RAPD profile were found for all strains examined, with buffer 9 giving the clearest and most discriminatory profiles. These results demonstrate the importance of the optimisation of PCR reactions. As demonstrated by other workers [15], variation in the magnesium concentration resulted in marked alterations of profiles. Magnesium promotes and stabilises primer–template interactions, has an effect on denaturation of the template DNA and is required for enzyme activity and fidelity. High concentrations of magnesium may inhibit amplification due to inadequate denaturation of the template DNA and can also lead to the accumulation of non-specific amplification products. By contrast, insufficient magnesium ions will reduce yield as primers are unable to anneal efficiently to the template DNA [16]. Potassium chloride can also affect PCR specificity as it facilitates primer annealing [16] and can directly affect Taq polymerase [17]. A lower concentration of KCl in the PCR buffer was optimal for the primer–template combination used in this study. Profiles produced using a buffer with a high concentration of potassium chloride resulted in smeared profiles. This may have occurred due to extension of one primer without extension of a primer on the opposite strand. The pH of the PCR buffer had little effect on the profiles obtained; however, if the pH is too low, non-specific reactions can occur and if too high, yield is reduced. Buffer 9 (1.5 mM MgCl₂, 25 mM KCl, pH 9.2) was selected for use in further RAPD reactions.

3.2. RAPD optimisation – template DNA concentration

When optimising RAPD the template concentration can be a critical factor to consider. The concentration of DNA can influence the number of products resulting in different fingerprints, therefore standardisation of the template concentration is important for reproducibility. Excess template can result in suppression of the amplification process due to competition between template DNA and first-round amplicons and relative shortage of primers. Fig. 2 shows the RAPD profiles obtained using buffer 9 with template concentrations ranging from 3–40 ng µl⁻¹. The profiles remained constant throughout this concentration range and a DNA concentration of 10 ng µl⁻¹ with buffer 9 was chosen as the optimised RAPD system for analysis of clinical isolates.

3.3. Optimised RAPD of P. acnes isolates

Strains of P. acnes isolated from various sources were examined using the optimised RAPD method. Fig. 3 shows the profiles from four strains chosen to illustrate the different profiles, containing six to eight bands in the 200–1500 bp range. All isolates were typeable by this method and, on repeated testing on two separate occasions three months apart, all profiles could be assigned
to the same pattern as that previously generated giving 100% intra- and inter-reproducibility (Fig. 3). The optimised RAPD protocol was highly discriminatory (DI, 0.98), in keeping with the requirement for an index greater than 0.9, which is desirable if the results of a typing scheme are to be interpreted with confidence [14].

The relationship between \( P. acnes \) profiles was determined by UPGMA cluster analysis (Fig. 4). Clinical isolates of \( P. acnes \) were represented in a major cluster at 65% similarity, which further subdivided into two distinctive profile types. One of these distinctive profile types contained NCTC 737, a \( P. acnes \) serotype I strain [18], and the other contained NCTC 10390, of serotype II (S. Patrick, personal communication). It will be interesting to determine whether the clinical strains in the two major RAPD groups belong to serotype I or II and hence if this typing system distinguishes between the two serotypes. In particular, the cluster containing the serotype I strain, NCTC 737, was characterised by the presence a band of 200 bp (e.g. strain a in Fig. 3). Studies are currently in progress to sequence this band as a guide to the genetic differences between the two serotypes of \( P. acnes \). A DNA probe based on this sequence might provide an alternative approach to the identification of type I and type II strains.

The RAPD system differentiated between \( P. acnes \) and the related \( Propionibacterium \) species, \( P. granulosum \) and \( P. avidum \), which gave markedly different profiles with less than 40% similarity to those of \( P. acnes \). Strains isolated from different clinical sources were distributed amongst the RAPD profile types within the \( P. acnes \) clusters. Fu-
ture work with more strains from a larger variety of clinical sources will enable us to determine whether certain genotypes are associated with specific clinical conditions and phenotypic properties.

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References

[1] Brook, I. and Frazier, E.H. (1991) Infections caused by Propionibacterium species. Rev. Infect. Dis. 13, 819-822.
[2] Burk hart, C.G., Burk hart, C.N. and Lehmann, P.F. (1999) Acne: a review of immunologic and microbiologic factors. Postgrad. Med. J. 75, 328-331.
[3] Deramo, V.A. and Ting, T.D. (2001) Treatment of Propionibacterium acnes endophthalmitis. Curr. Opin. Ophthalmol. 12, 225-229.
[4] Vandenbos, F. et al. (2001) Ventricular patch endocarditis caused by Propionibacterium acnes: advantages of gallium scanning. J. Infect. 43, 249-251.
[5] Sulkowski, M.S., Abolnik, L.Z., Morris, E.I. and Granger, D.L. (1994) Infectious arthritis due to Propionibacterium acnes in a prosthetic joint. Clin. Infect. Dis. 19, 224-225.
[6] El shi, Y. et al. (2002) Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. J. Clin. Microbiol. 40, 198-204.
[7] Tunney, M.M., Patrick, S., Gorman, S.P., Nixon, J.R., Anderson, N., Davis, R.I., Hanna, D. and Ramage, G. (1998) Improved detection of infection in hip replacements. A currently underestimated problem. J. Bone Joint Surg. Br. 80, 568-572.
[8] Stirling, A., Worthington, T., Rafiq, M., Lambert, P.A. and Elliott, T.S. (2001) Association between sciatica and Propionibacterium acnes. Lancet 357, 2024-2025.
[9] Rossi, F., Torriani, S. and Dellaglio, F. (1998) Identification and clustering of dairy propionibacteria by RAPD-PCR and CGE-REA methods. J. Appl. Microbiol. 85, 956-964.
[10] Ting, T.D. et al. (1999) Evaluation of Propionibacterium acnes isolates using contour-clamped homogeneous electric field gel electrophoresis. Anaerobe 5, 579-582.
[11] Tipper, J.L., Eady, E.A., Cove, J.H. and Cunliffe, W.J. (1993) A method for lysing cutaneous propionibacteria and its use to clone antibiotic determinants from Propionibacterium acnes. Br. J. Dermatol. 129, 488-489.
[12] Tyler, K.D., Wang, G., Tyler, S.D. and Johnson, W.M. (1997) Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. J. Clin. Microbiol. 35, 339-346.
[13] Hilton, A.C., Banks, J.G. and Penn, C.W. (1996) Random amplification of polymorphic DNA (RAPD) of Salmonella: strain differentiation and characterization of amplified sequences. J. Appl. Bacteriol. 81, 575-584.
[14] Hunter, P.R. and Gaston, M.A. (1988) Numerical index of the discriminability of typing systems: an application of Simpson’s index of diversity. J. Clin. Microbiol. 26, 2465-2466.
[15] Ellsworth, D.L., Rittenhouse, K.D. and Honeycutt, R.L. (1993) Artificial variation in randomly amplified polymorphic DNA banding patterns. Biotechniques 14, 214-217.
[16] Hopkins, K.L. and Hilton, A.C. (2001) Optimization of random amplification of polymorphic DNA analysis for molecular subtyping of Escherichia coli O157. Lett. Appl. Microbiol. 32, 126-130.
[17] Hilton, A.C., Banks, J.G. and Penn, C.W. (1997) Optimization of RAPD for fingerprinting Salmonella. Lett. Appl. Microbiol. 24, 243-248.
[18] Johnson, J.L. and Cummins, C.S. (1972) Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria, and strains of Arachnia propionica. J. Bacteriol. 109, 1047-1066.
[19] Schoettlin, W., Neilson, K.B. and Mathur, E. (1994) Optimisation of PCR using the opti-prime kit. Stateg. Mol. Biol. Newsl. 6, 43-44.