Rapid differentiation of *Prevotella intermedia* and *P. nigrescens* by 16S rDNA PCR-RFLP

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Prevotella intermedia and the newly described *P. nigrescens* cannot be reliably distinguished by phenotypic tests. In this study, restriction endonuclease digestion of amplified 16S rDNA (16S rDNA PCR-RFLP) was used to generate restriction profiles of the type strains of *P. intermedia* and *P. nigrescens* and 43 fresh isolates identified as belonging to one of the two species. Whole-cell protein profiles were obtained by SDS-PAGE for comparative purposes. The type strains of *P. intermedia* and *P. nigrescens* were easily distinguished by 16S rDNA PCR-RFLP and the fresh isolates were assigned to either species on the basis of their restriction profiles. The identifications obtained were identical to those obtained by protein profiles. 16S rDNA PCR-RFLP is a rapid and reliable method for the differentiation of *P. intermedia* and *P. nigrescens*.

Introduction

Members of the genus *Prevotella* are gram-negative anaerobic bacilli commonly isolated from the human mouth and have been associated with various oral infections [1]. *P. nigrescens* is a recently described species closely related to *P. intermedia* and was described previously as *P. intermedia* serotypes 2 and 3 [2]. *P. intermedia* and *P. nigrescens* can be distinguished by multi-locus enzyme electrophoresis, protein profiles and monoclonal antibodies [3, 4]. Unfortunately, there are no reliable phenotypic tests that differentiate between the two species. The methods mentioned above are relatively time consuming procedures and results can be difficult to interpret.

Restriction endonuclease analysis of amplified 16S rRNA genes (16S rDNA PCR-RFLP) has been shown to be a useful typing technique for a number of groups of organisms, and within some genera can be used to identify species [5, 6].

In this study, reference strains of *P. intermedia* and *P. nigrescens* and strains previously identified as '*P. intermedia*' (without differentiation of *P. nigrescens*) were studied by 16S rDNA PCR-RFLP to assess the usefulness of the method for the discrimination of the two species. SDS-PAGE protein profiles were also generated from the strains for comparative purposes.

Materials and methods

Bacterial strains

The strains studied are listed in Table 1 together with their site of isolation. Clinical isolates were identified as *P. intermedia/nigrescens* on the basis of their black pigmentation on blood agar, production of indole and fermentation of glucose but not lactose or sucrose by methods described previously [7].

16S rRNA PCR-RFLP

Bacterial cultures were maintained on Fastidious Anaerobe Agar (FAA, Lab M) supplemented with sheep blood (TCS) 5% in an atmosphere of N₂ 80% H₂ 10% and CO₂ 10%. Genomic DNA was prepared from the strains by standard methods [8]. 16S rRNA gene sequences were amplified by the polymerase chain reaction (PCR) with primers 27f and 1492r [9] and *Taq* polymerase (Boehringer Mannheim) according to the manufacturer's instructions, except that MgCl₂ was adjusted to 2.5 mM. There were 35 cycles, each consisting of template denaturation for 1 min at 94°C, primer annealing for 1 min at 52°C and extension for 2 min at 72°C. Amplified 16S rRNA gene sequences were digested with restriction endonucleases *Cfo*I (Promega), *Taq*I (New England Biolabs) and *Hinf*I (New England Biolabs) according to the manufacturer's instructions. Digestion products were separated on agarose gels 2%, stained with ethidium bromide and photographed under UV light.

Table 1. Strains included in the study and their identification

Strain no.	Site of isolation	Identification
ATCC 33563	Vincent's gingivitis	<i>P. nigrescens</i>
ATCC 25611	Empyema	<i>P. intermedia</i>
ATCC 25261	Laryngotomy wound	<i>P. nigrescens</i>
W2129	Peri-apical abscess	<i>P. nigrescens</i>
W2169	Retained root	<i>P. nigrescens</i>
W2207	Periodontal abscess	<i>P. intermedia</i>
W2246	Peri-apical abscess	<i>P. intermedia</i>
W2272	Peri-apical abscess	<i>P. nigrescens</i>
W2399	Peri-apical abscess	<i>P. nigrescens</i>
W2567	Periodontal abscess	<i>P. nigrescens</i>
W2613	Peri-apical abscess	<i>P. nigrescens</i>
W2758	Retained root	<i>P. nigrescens</i>
W1669	Pericoronitis	<i>P. nigrescens</i>
W1696	Pericoronitis	<i>P. nigrescens</i>
W1790	Pericoronitis	<i>P. nigrescens</i>
W729	Periodontal pocket	<i>P. intermedia</i>
W793	Periodontal pocket	<i>P. intermedia</i>
W975	Periodontal pocket	<i>P. intermedia</i>
W1181	Periodontal pocket	<i>P. intermedia</i>
W1352	Periodontal pocket	<i>P. intermedia</i>
W1380	Periodontal pocket	<i>P. intermedia</i>
W1410	Periodontal pocket	<i>P. intermedia</i>
W2708	Periodontal pocket	<i>P. nigrescens</i>
W2709	Periodontal pocket	<i>P. nigrescens</i>
W2753	Periodontal pocket	<i>P. nigrescens</i>
W2763	Periodontal pocket	<i>P. intermedia</i>
W2834	Periodontal pocket	<i>P. intermedia</i>
W2835	Periodontal pocket	<i>P. intermedia</i>
W2839	Periodontal pocket	<i>P. nigrescens</i>
W2843	Periodontal pocket	<i>P. nigrescens</i>
W2851	Periodontal pocket	<i>P. intermedia</i>
W2857	Periodontal pocket	<i>P. nigrescens</i>
W2860	Periodontal pocket	<i>P. nigrescens</i>
W2867	Periodontal pocket	<i>P. nigrescens</i>

Protein profiles

Bacteria were harvested from 48-h FAA cultures and prepared for SDS-PAGE as described previously [10]. SDS-PAGE was performed on gradient gels 10–15% by means of the PhastSystem (Pharmacia) [10].

Results

Protein profiles for the three reference strains and three unknown strains are shown in Fig. 1. Overall, the banding patterns were similar but there were some key

Fig. 1. Protein profiles of *P. intermedia* and *P. nigrescens*. Lanes: A, C, F, *P. intermedia* strains W2835, W793, ATCC 25611; B, D, E, *P. nigrescens* strains W2860, ATCC 33563, ATCC 25261, M, mol. wt markers, 205, 116, 97, 84, 66, 55, 45, 36, 29, 24, 20, 14.2 and 6.5 kDa.

distinguishing features. *P. nigrescens*- specific proteins were of mol. wts 31, 38, 62 and 100 kDa whereas 44- and 58-kDa proteins were typical of *P. intermedia*. On this basis, 13 of the 31 unknown strains were assigned to *P. intermedia*.

RFLP patterns for the 16S rDNA PCR products from the reference and three unknown strains, obtained with the three enzymes, are shown in Fig. 2. Clear differences in the profiles for the reference strains of *P. intermedia* and *P. nigrescens* were found for all enzymes and, therefore, unknown strains were assigned to one or the other of the species on that basis. There was complete agreement between the identifications based on protein profiles and RFLP patterns. *P. intermedia* was associated more commonly with periodontitis (11 of 19 isolates) than with acute oral abscesses (2 of 9 isolates) (Table 1).

Discussion

The recent improvements in the taxonomy of oral bacteria due to the introduction of new molecular methods has allowed more specific associations to be made between individual species and disease states. This was confirmed in the present study, where *P. intermedia* was associated with periodontitis, whereas *P. nigrescens* was more common in oral abscesses. However, the absence of reliable phenotypic characters to distinguish between related species makes identification difficult in large-scale studies and for small laboratories. This study has confirmed that SDS-PAGE can be used to distinguish *P. intermedia* and

M A B C D E F G H I J K L N O P Q R S M

Fig. 2. Amplified 16S rDNA from *P. intermedia* and *P. nigrescens* digested with *Cfo*I (lanes A–F), *Hin*fl (G–L) and *Taq*I (N–S). Lanes: A, C, D, G, I, J, N, P, Q, *P. nigrescens* strains ATCC 33563, ATCC 25261, W2860; B, E, F, H, K, L, O, R, S, *P. intermedia* strains ATCC 25611, W2207, W1181; M, mol. wt markers — 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 220 and 154 bp.

P. nigrescens but, as has been seen, the protein profiles obtained are rather similar and the possibility of mis-identification, although not encountered in this study, exists. However, 16S rDNA PCR-RFLP provides a definitive and simple method to differentiate these species. Conventional ribotyping, involving hybridisation of 16S and 23S rRNA probes with digested genomic DNA, often generates too many different ribotypes within a species to allow confident species identification. By contrast, the method employed here (amplification of the 16S rDNA alone), produced consistent patterns for the two species and, proved suitable for identification purposes. The PCR is now a routine method in many laboratories and this, linked to rapid DNA extraction methods, allows the procedure described here to be performed within 1 day. Therefore, we conclude that 16S rDNA PCR RFLP is a rapid and reliable method for distinguishing oral isolates of *P. nigrescens* and *P. intermedia* that may be applied confidently to large-scale studies.

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