ORAL MICROBIOLOGY AND IMMUNOLOGY

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Short communication

# *fimA* genotypes and PFGE profile patterns in *Porphyromonas gingivalis* isolates from subjects with periodontitis

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*Perez-Chaparro PJ, Rouillon A, Minet J, Lafaurie GI, Bonnaure-Mallet M.* fimA *genotypes and PFGE profile patterns in* Porphyromonas gingivalis *isolates from subjects with periodontitis.*
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**Background and objectives:** *Porphyromonas gingivalis* is frequently identified to type by evaluation of *fimA* polymorphisms and less often by pulsed-field gel electrophoresis (PFGE) because of the technical intricacies of PFGE. To compare these techniques, we genotyped *P. gingivalis* clinical isolates as to (i) their *fimA* type and (ii) their whole genome restriction profile (PFGE analysis).
**Material and methods:** Thirty-two *P. gingivalis* strains were isolated from 16 unrelated periodontitis patients. Two strains were isolated from each patient. Strains were subjected to a *fimA*-typing polymerase chain reaction (PCR) assay. Strains that could not be typed by PCR were submitted to sequencing of the entire *fimA* gene. The PFGE profiles of clinical strains were compared using bioinformatic analysis.
**Results:** Seven of the 32 isolates were not typeable by PCR and so their entire *fimA* gene was sequenced. The sequencing identified each strain as belonging to a single *fimA* type. In one case, sequencing of the *fimA* gene did not agree with the result obtained using *fimA* PCR typing. With the exception of one patient, each patient presented isolates bearing the same *fimA* type. However, in three patients, isolates with the same *fimA* type presented different PFGE pulsotypes.
**Conclusion:** The *P. gingivalis* typing using *fimA* PCR has limitations in typeability and discriminatory power. A typing technique for *P. gingivalis* that is easy to perform but that presents adequate typeability and discriminatory power is needed if we want to better understand the epidemiology of periodontal disease.

Key words: *Porphyromonas gingivalis*; *fimA*, pulsed-field gel electrophoresis; genotyping; periodontitis

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Periodontitis is an infectious disease caused by microorganisms that colonize and invade periodontal tissue, destroying connective tissue attachment and alveolar bone. *Porphyromonas gingivalis* is a gram-negative, anaerobic, black-pigmented rod bacterium and is considered to be an opportunistic periodontal pathogen; it has been described as a major etiological agent of chronic destructive periodontitis that is involved in both the onset and progression of disease (6, 12). The *fimA* gene encodes the subunit protein of the fimbria, fimbrillin, which ranges in size from 41 to 49 kDa (2, 8, 9). Based on nucleotide sequences from reference and clinical strains, six variants (I–V and Ib) of the *fimA* gene have been described. A polymerase chain reaction (PCR) typing technique based on the characterization of *fimA* polymorphism has been developed elsewhere (2). This latter technique is frequently used for the characterization of *P. gingivalis* strains; however, other typing techniques such as pulsed-field gel electrophoresis (PFGE) have been used for *P. gingivalis* genotyping. PFGE has been employed to determine the genetic relatedness between *P. gingivalis* isolates (7).

To study the genetic relatedness of 32 *P. gingivalis* clinical isolates obtained from patients with periodontitis, a PCR-based *fimA*-typing technique and PFGE were used on all strains, as well as sequencing of the entire *fimA* gene in some strains. Furthermore, to study the relationship between *fimA* genotyping and PFGE, we compared the results obtained with the *fimA* PCR-based genotyping with the results obtained with PFGE. *P. gingivalis* strains were collected from 16 patients suffering from periodontitis as previously described (11). For the majority of the patients (13 out of 16) at least two strains were isolated from the same patient. All strains were previously identified as *P. gingivalis* and confirmed by routine laboratory methods. Single colonies from each sample were grown on plates under anaerobic conditions as previously described (11).

Single colony lysates were submitted to a PCR assay for *fimA* genotyping using the six primer sets designed by Amano et al. (1, 2) and Nakagawa et al. (8, 9), using amplification conditions previously described by Amano et al. (1, 2). *P. gingivalis* strains ATCC33277 (*fimA* type I), OMZ409 (provided by Prof. Gmür, Zurich; *fimA* type II), TNF (*fimA* type III), ML2G (*fimA* type Ib), and W83 (*fimA* type IV) were included as standard reference strains. Each experiment was repeated three times.

The *fimA* PCR genotyping of seven of the 32 strains were positive for more than one primer set and therefore non-typeable (Table 1). Considering that the *fimA* gene is present in a single copy on the *P. gingivalis* chromosome (3, 5) and that PCRs were performed using DNA from single colonies instead of DNA from pooled samples, amplification of the entire *fim*A gene was undertaken using the universal primer set M1 and M2 and applying the conditions previously described by Fujiwara et al. (5). Sequencing was performed using the same M1 and M2 primers and the BigDye® Terminator v3.1 cycle sequencing kits following the manufacturer's instructions (Applied Biosystems, CA, USA; http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_041329.pdf). The results were analysed by an ABI PRISM 3100 Genetic analyzer (Applied Biosystems, Courtaboeuf, France). Nucleotide sequence fragments of each clinical strain were made using the Contigexpress tool available within the Vector NTI advance 10 software (Invitrogen, CA, USA). The DNA sequences obtained, including those of the six *fimA* type reference strains, were used for multiple alignment using the CULSTALW2 online software (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The following reference strain sequences from the GenBank database were used: ATCC33277 (ID17795-I), OMZ409 (D17799-II), BH6/26 (D17801-III), W83 (AE015924-IV), HNA 99 (AB027294-V), and HG1691 (AB058848-Ib). Subsequently, a cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) based on a Pearson coefficient. Results of analyses of the nucleotide sequences of reference strains and of

*Table 1. fimA* profiles of *Porphyromonas gingivalis* strains investigated by polymerase chain reaction

| | In subgingival plaque sample | | | PCR | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | Inclusion study sample | Before scaling and root planing | In blood sample | *fimA* type | *fimA*I | *fimA*Ib | *fimA*II | *fimA*III | *fimA*IV | *fimA*V | Assigned *fimA* type after sequencing |
| 1 | NA | UIBO 655 | | Unclassifiable | − | NI | + | − | + | − | IV |
| | | | UIBO 655 H3 | Unclassifiable | − | NI | + | − | + | − | IV |
| | | | UIBO 655 H4 | Unclassifiable | + | − | + | − | + | − | IV |
| 2 | NA | UIBO 695 | | Unclassifiable | − | NI | + | − | + | − | II |
| | | | UIBO 695 H2 | II | − | NI | + | − | − | − | NS |
| 3 | NA | UIBO 728 | | III | − | − | − | + | − | − | NS |
| | | | UIBO 728 H3 | III | − | − | − | + | − | − | NS |
| 4 | UIBO 735 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 771 | | II | − | NI | + | − | − | − | NS |
| | | | UIBO 771 H2 | II | − | NI | + | − | − | − | NS |
| 5 | UIBO 751 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 801 | | II | − | NI | + | − | − | − | NS |
| | | | UIBO 801 H3 | II | − | NI | + | − | − | − | NS |
| 6 | NA | UIBO 1047 | | Ib | − | + | − | − | − | − | NS |
| | | | UIBO 1047 H3 | Ib | − | + | − | − | − | − | NS |
| 7 | NA | | UIBO 486H2 | III | − | − | − | + | − | − | NS |
| | | | UIBO 486 H3 | III | − | − | − | + | − | − | NS |
| 8 | NA | UIBO 482 | | Ib | − | + | − | − | − | − | NS |
| 9 | NA | UIBO 472 | | Unclassifiable | + | − | + | − | − | − | III |
| 10 | NA | UIBO 710 | | II | − | NI | + | − | − | − | NS |
| 11 | UIBO 456 | | | Ib | − | + | − | − | − | − | NS |
| | | UIBO 465 | | Ib | − | + | − | − | − | − | NS |
| 12 | UIBO 712 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 742 | | Unclassifiable | − | NI | + | − | + | − | II |
| 13 | UIBO 507 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 537 | | Unclassifiable | − | NI | + | − | + | − | IV |
| 14 | UIBO 724 | | | I | + | NI | − | − | − | − | NS |
| | | UIBO 760 | | I | + | NI | − | − | − | − | NS |
| 15 | UIBO 741 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 783 | | II | − | NI | + | − | − | − | NS |
| 16 | UIBO 392 | | | II | − | NI | + | − | − | − | NS |
| | | UIBO 421 | | II | − | NI | + | − | − | − | NS |

+, positive PCR amplification; −, negative PCR amplification; NI, PCR primer set not indicated; NS, not sequenced; NA, not able to process the sample.

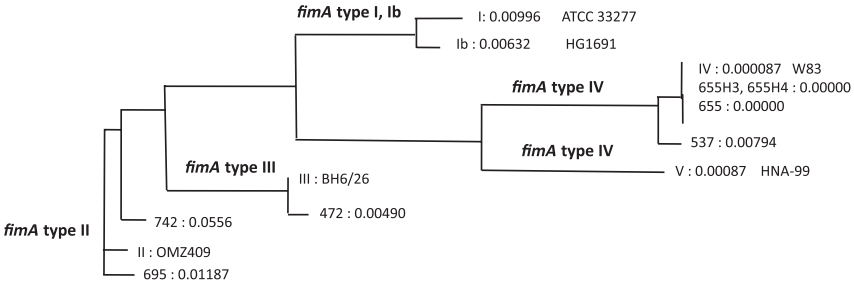


*Fig. 1.* Phylogram of *Porphyromonas gingivalis* isolates and *fimA* reference strain. Cluster analysis was performed with the unweighted pair group method with arithmetic mean based on a Pearson coefficient. Phylogram is between the *fimA* nucleotide sequences of reference strains and clinical isolates.

clinical isolates are presented in Fig. 1 as a phylogram. A single *fimA* type was assigned to each strain (Table 1). That the phylogenetic tree representation is different from the one published by Enersen et al. (4) may be the result of differences in the sequences obtained. Enersen et al. (4) considers the sequence 5′-GTG-3′ as the translational start codon whereas we considered the *fimA* gene translational start codon as being 5′-ATG-3′. These two codons are in-frame and separated by 108 nucleotides in the type I *fimA* gene. This discrepancy yields proteins that are 36 amino acids shorter. The 5′-ATG-3′ codon for *fimA* has been previously described by Park et al. (10). Moreover, the software used for the phylogenetic analyses in our study and in that by Enersen et al. (4) were different.

In six of the seven cases the *fimA* type corresponded to one of the two types previously amplified by PCR. However, the *fimA* sequence of the strain obtained from Patient 9 (UIBO 472) was type III whereas the PCR assay was positive for *fimA* types I and II. Enersen et al. have already described this non-correlation between *fimA* PCR typing and sequencing (4).

PFGE profiles of 32 isolates were submitted to bioinformatics analysis using the GelCompar software package (Applied Maths, Kortrijk, Belgium). Cluster analysis was performed with the UPGMA analysis based on a Dice coefficient, as previously described (11). Results can be seen on a dendogram in Fig. 2. Two isolates were considered as genetically related if their Dice coefficient was ≥85%. Comparison between results of *P. gingivalis fimA* genotypes and *P. gingivalis* PFGE typing are presented in Fig. 2.

Results show that in most cases (10/13) the *P. gingivalis* strains isolated from a single patient showed the same PFGE profile, indicating that the isolates belong

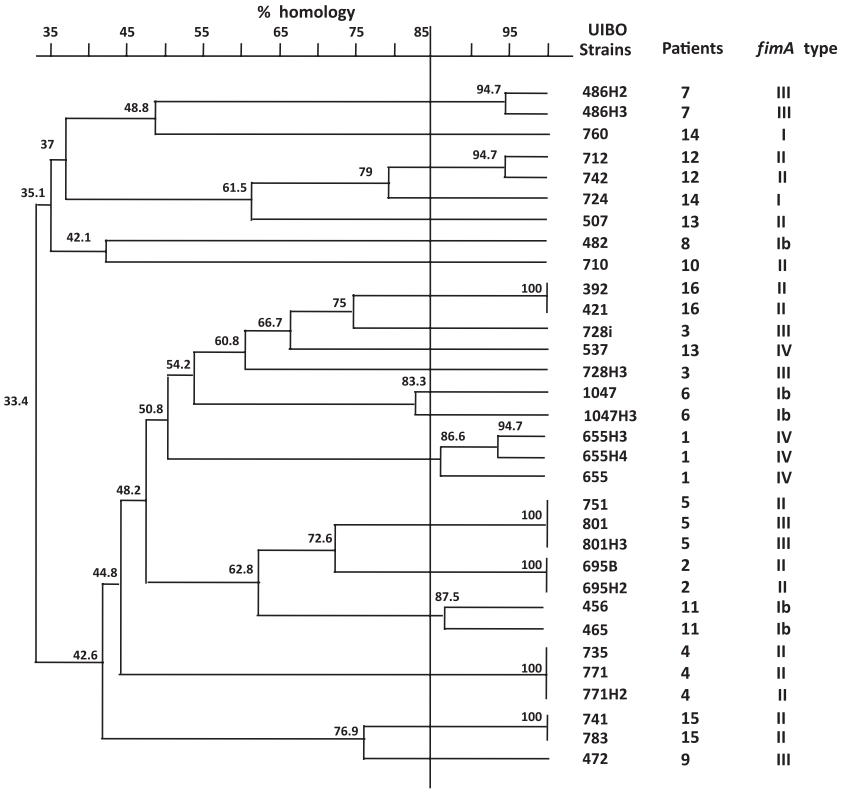


*Fig. 2.* Dendogram representing the genetic relationships between 32 strains of *Porphyromonas gingivalis*. Cluster analysis was performed with unweighted pair group method with arithmetic mean analysis based on a Dice coefficient. For a Dice coefficient ≥85%, the isolates were considered to be genetically related. Strains assigned *fimA* type after PCR *fimA* genotyping and *fimA* sequencing are included.

to the same clone. Nevertheless, in three patients (Patients 3, 6, and 14) different *P. gingivalis* clones of identical *fimA* type were obtained. Each time *fimA* genotyping resulted in more than one amplification (positive for types I, II, and IV; or positive for types II and IV), a primer (reverse) common to all the types that were positive was involved. This indicates the limitation of *fimA* typing by PCR. Therefore, *fimA* PCR genotyping of *P. gingivalis* should be reconsidered. *fimA* genotyping does not present the same discriminatory power as PFGE and should not be used to infer clonality in *P. gingivalis*.

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