Review

Genetic diversity of *Porphyromonas gingivalis* and its possible importance to pathogenicity

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Özmeriç N, Preus HR, Olsen I. Genetic diversity of *Porphyromonas gingivalis* and its possible importance to pathogenicity. Acta Odontol Scand 2000;58:183–187. Oslo. ISSN 0001-6357.

During recent years much effort has been put into understanding the genetic composition of the oral populations of black-pigmented anaerobic bacteria. One of them, *Porphyromonas gingivalis*, is a putative periodontopathogenic organism considered to be particularly relevant in the etiology of adult periodontitis. It has been shown in studies using molecular typing methods that most bacterial populations consist of numerous genetic clones, and that only a small proportion of these clones cause disease. Elucidation of a possible association of genotypic profiles with either disease or clinical healthy condition is important for understanding the pathogenic characteristics of bacteria. Studies addressing this issue as it relates to *P. gingivalis* are reviewed in the present article. Genotypic characterization of *P. gingivalis* strains has revealed extensive heterogeneity in natural populations of this bacterium. Some of the potential virulence factors of *P. gingivalis* have been purified and cloned and methods have been established to identify their genes. Although no studies have clearly defined the relationship between a specific genotype of *P. gingivalis* and periodontal status of the host, it seems that molecular typing tools, which are undergoing rapid improvements, will allow us to distinguish between virulent and avirulent strains of the same species in the near future. □ *Genetic heterogeneity; Porphyromonas gingivalis; virulence*

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Porphyromonas gingivalis is an anaerobic, asaccharolytic, gram-negative coccobacillary rod that is widely considered as a major pathogen in the development of destructive periodontal diseases such as severe adult periodontitis (1–10). *P. gingivalis* exhibits a number of virulence factors, including fimbrial adhesins, lipopolysaccharide (LPS), capsule, collagenase, and cysteine proteinases with trypsin-like activity (11–16). In the human oral cavity, only *P. gingivalis* strains appear capable of degrading type I collagen (17). Adherence of *P. gingivalis* to supragingival plaque or gingival tissue surfaces is probably an initial step in the pathogenesis of adult periodontitis. *P. gingivalis* also adheres to other bacteria, red blood cells and collagen complexes (18–20). It was demonstrated that fimbriae play a direct role in the adhesion of this bacterium to *Actinomyces viscosus* (20). *P. gingivalis* has also been shown to invade human gingival epithelial cells (21).

P. gingivalis-associated virulence factors cause proteolysis, development of edema, neutrophil accumulation at infected sites as well as bleeding at probing which are hallmarks of periodontitis (22). Therefore, it is important to identify the virulence factors used by this organism to initiate tissue destruction in the periodontium.

In recent years it has become evident that for many common chronic diseases there are modifying factors that amplify some disease mechanisms to make the clinical condition more severe. Differences in the progression rate of an infection are most likely due to variance in the virulence of strains within the pathogenic species, although host mechanisms should not be excluded.

In order to identify individual isolates of *P. gingivalis*, biotyping and serotyping have been used for several years. Six serogroups of *P. gingivalis* based on capsular antigens which have been linked to pathogenicity in an animal model are currently recognized, and two biotypes have been identified on the basis of the presence or absence of catalase activity (23–25). Other potential techniques to characterize individual isolates include whole-cell protein profiles after sodium dodecyl sulphate polyacrylamide gel electrophoresis and subtyping based on outer membrane proteins (OMPs) or LPS profiles.

These techniques are likely to have only limited application for assessment of the extent of heterogeneity of strains and for ecological and epidemiological studies of *P. gingivalis* (25). Therefore, the probability of any two unrelated isolates having identical biotype, serotype, OMP, or LPS pattern is large.

Obviously, there has been a need to establish alternative and more discriminative test methods to screen epidemiologically group-related strains within species and to establish a correlation with pathogenicity.

Since genetic diversity among isolates is a reflection of sequence differences in their chromosomes, molecular typing methods such as restriction endonuclease analysis

(REA) (26, 27), restriction fragment length polymorphism (RFLP) (28), multilocus enzyme electrophoresis (MEE) (29), and arbitrarily primed polymerase chain reaction (AP-PCR) (30) might be sensitive tools for genetic characterization of different strains.

The aim of the present paper is to review studies based on molecular typing methods which have investigated the genetic heterogeneity of *P. gingivalis* with respect to its pathogenic features.

Pathogenic features of different strains of *P. gingivalis*

Strains of *P. gingivalis* may differ considerably with respect to pathogenic properties, and various strains have been classified as pathogenic and non-pathogenic, or as invasive and non-invasive (31–33). These classifications were mainly based on animal studies which showed differences in proteolytic activity, and the ability of strains to cause spreading of infection in mice or guinea pigs. Monoinfection of gnotobiotic rats with *P. gingivalis* induces alveolar bone loss (34), and inoculation of this microorganism in the subgingivae of monkeys exacerbates periodontal breakdown (35). In the mouse model, certain strains are highly invasive and can produce fatal phlegmonous abscesses (36, 37). Other strains cause non-lethal, localized abscesses in mice. In one study, the periodontopathic potential of two strains of *P. gingivalis* was compared in germ-free rats (38). Although one strain was not more periodontopathic than the other, the pattern of periodontal destruction was different between the two strains. One strain caused horizontal bone destruction, whereas the other strain induced vertical bone loss. The reason why these strains caused varying patterns of destruction is unknown, but the authors concluded that certain *P. gingivalis* strains have unique abilities to cause diseases.

Host responses towards different strains of *P. gingivalis* vary greatly. Thus, strain variability has been observed in the resistance of *P. gingivalis* to phagocytosis (39, 40).

Some of the potential virulence factors of *P. gingivalis* have been purified and cloned (41–43). The genes encoding key enzymes and structural components of *P. gingivalis* have been discussed extensively in a recent article (44). In one of these studies, the prevalence of the collagenase gene in *P. gingivalis* strains was determined by PCR. Of 21 *P. gingivalis* isolates from plaque samples and one reference strain of *P. gingivalis*, a total of 16 isolates were confirmed as positive for the collagenase gene (*prtC*) using DNA hybridization with a digoxigenin-labeled *prtC* PCR product as a probe. Forty-eight strains of 16 other bacterial species from oral and extraoral infections were similarly analyzed by PCR, but no fragment bands were found. In 12 of the 16 *prtC* positive strains, the restriction analysis of PCR products revealed fragment patterns identical to the known sequence, and in the remaining strains, four distinct patterns were found (45). A considerable heterogeneity in the gene encoding the fimbrillin subunit of *P. gingivalis* strains has been observed and it was suggested that such differences might be important in the function and immunogenicity of the fimbriae (28).

The conservation of a principal arginine-specific protease gene, *prpR1*, of *P. gingivalis*, and inter-strain heterogeneity within *prpR1* in 38 isolates of *P. gingivalis* has been examined using Southern hybridization with a probe to the catalytic (α) domain of the *prpR1* gene and the amplification of the α domain by PCR and REA of the amplicon (46). This gene was highly conserved in commonly used laboratory strains and in a selection of clinical isolates of *P. gingivalis*. Three genotypes were demonstrated with respect to *prpR1* and with the exception of one patient, all patients yielded only a single type (46).

Genetic diversity of *P. gingivalis*

The bacterial flora of the mouth is among the most diverse of any of the resident floras associated with humans. Some oral species can be represented by several individual strains in a host, while in others a single clone can represent one species (47, 48). In a study with a large series of *P. gingivalis* strains from different continents, any specific clonal type with a predominant distribution over the globe, over a continent, over a country or even locally could not be observed (49).

Discrimination of microorganisms by a detectable phenotype implies that the organisms should also be distinguishable at the DNA level, and DNA has become the most popular target of molecular typing methods (Table 1). The use of such methods for assessing intrafamilial transmission of black-pigmenting bacteria was recently reviewed by Özmeriç *et al.* (50). AP-PCR has the ability to detect highly specific DNA profiles with no prerequisite for knowing the DNA sequences, and the utility of AP-PCR for the clonal analysis of *P. gingivalis* clinical isolates has been investigated (51). Twenty 10-base oligonucleotide primers were tested and it was found that two primers produced clear and distinct DNA bands. These two primers were selected for analysis of 73 *P. gingivalis* strains. While on the basis of the primer GACCGCTTGT, 73 strains were classified into 23 genotypes; the other primer AGGGGTCTTG distinguished 45 genotypes (51).

Thirty-three isolates of *P. gingivalis* obtained from dental patients either with periodontitis or with infected root canals were analysed by REA (26). The majority of independent *P. gingivalis* isolates had a unique DNA fingerprint indicating extensive genetic heterogeneity within this species. Only a single REA type of *P. gingivalis* generally colonized individuals and the interindividual strains were genetically different (26, 52, 53).

Hybridization probes derived from the fimbrial locus of *P. gingivalis* strain FDC 381 have been used to examine RFLPs at the fimbrillin locus in 39 human and animal *P. gingivalis* strains (28). Twenty-five distinct RFLP patterns

Table 1. Studies on clonal diversity in *P. gingivalis*

	No. of strains	Molecular typing method	No. of clones	Source of strains
Loos et al. (26)	33	REA*	29	Human, laboratory
Loos & Dyer (28)	39	RFLP†	25	Human, animal
Ménard et al. (30)	9	AP-PCR‡	9	Laboratory
Socransky & Martin (54)	25	Ribotyping	6	Human
Loos et al. (29)	100	Multilocus enzyme electrophoresis	78	Human, animal, laboratory
Ménard & Mouton (59)	16	AP-PCR	16	Human, animal
Saarela et al. (60)	31	Ribotyping	10	Human
Saarela et al. (61)	50	Ribotyping	9	Human
Petit et al. (62)	17	REA	9	Human
van Steenbergen et al. (52)	16	REA	10	Human
Madianos et al. (57)	64	REA	14	Animal
Chen & Slots (51)	73	AP-PCR	45	Human, laboratory, animal
Zhang et al. (58)	188	RFLP	5	Human
Ménard & Mouton (49)	97	AP-PCR	102	Human, laboratory, animal
Saarela et al. (63)	62	Ribotyping	4	Human
Asikainen et al. (64)	29	AP-PCR	27	Human
Teanpaisan et al. (56)	65	REA	10	Human
Ali et al. (55)	203	Ribotyping	60	Human, laboratory

* Restriction endonuclease analysis, † Restriction fragment length polymorphism, ‡ Arbitrarily primed polymerase chain reaction.

were revealed. The genetic variations of 100 human and animal strains were examined by determining the electrophoretic mobilities of 16 metabolic enzymes and the presence or absence of catalase activity (29). Seventy-eight electrophoretic types, which were further clustered into three major phylogenetic divisions, were identified. To investigate the clonality of *P. gingivalis* strains within the same host, 25 isolates from six subjects were analysed by means of ribotyping (54). Six distinct clonal types were detected. Each was unique for every subject. The genomic fingerprints of *P. gingivalis* strains isolated from saliva, tongue, and supragingival and subgingival plaque of nine subjects were also examined (47). It was found that eight subjects harbored one clonal type and in the subject harboring two clonal types, strains recovered from saliva and the gingiva belonged to a single fingerprint pattern, and strains recovered from the tongue and tonsils to another. While there is considerable heterogeneity among *P. gingivalis* isolates, intraindividual heterogeneity is low.

Studies on pathogenic bacteria have shown that clones of a single species can have different geographic distributions. A study using MEE indicated that identical genotypes of *P. gingivalis* could be recovered in patients from diverse geographical regions (29). Ribotyping was performed to examine the genetic relationships of *P. gingivalis* isolates of 52 periodontitis patients from different geographical locations. Apart from three patients, who harbored two ribotypes each, all subjects were colonized by only one ribotype (55).

Genetic diversity of *P. gingivalis* related to its pathogenicity

In order to achieve a better understanding of the considerable variability in virulence within *P. gingivalis* strains, the association of the genetic composition of bacteria related to disease has been addressed in a few studies. These have provided evidence that individual clones or genetic types may express different phenotypic characteristics. Each genetic variant may not express a unique phenotypic characteristic, and several may express the same characteristics. One could expect that pathogenic clones express specific phenotypic characteristics which confirm virulence. Therefore, it is reasonable to think that with pathogenic species where few clones cause disease, it should be possible to associate a single or limited number of these variants with a virulence factor.

The clonal types of black-pigmented anaerobes in relation to the periodontal disease status were investigated and it was found that nearly all subjects were colonized by a single, unique genotype of *P. gingivalis* and that the same type was present in both diseased and healthy sites (56). The high level of diversity and the absence of a predominant clonal type associated with periodontal disease suggest that all clonal types of *P. gingivalis* would be equally effective in colonizing the human host and that they share a common virulence potential (55).

A single periodontal pocket inhabited by *P. gingivalis* strains belonging to up to four different clonal types has been shown in an animal study and no clear association between clonal type and periodontal health status could be made (57).

One-hundred-and-eighty-eight *P. gingivalis* strains isolated from 13 periodontal pockets of eight periodontitis patients were investigated by using RFLP analysis. A total of five RFLP genotypes were identified. Four of the eight patients harbored only one RFLP genotype of *P. gingivalis*, and one patient harbored only two RFLP genotypes in different sites. In three other patients, multiple RFLP genotypes were found in single periodontal pockets (58).

On the other hand, no relationship could be established between genetic lineage on specific clusters of clones of *P. gingivalis* and the periodontal status of the host (29, 49). These findings suggested that interclonal variation in pathogenicity is relatively small.

As bacterial pathogenicity is dependent on both microbial and host factors, the determination of the nucleic acid sequence of strains of *P. gingivalis* associated with host response mediators, e.g., elevated antibody, could perhaps be beneficial to detect the causal relationship.

In conclusion, extensive genetic heterogeneity of *P. gingivalis* has been reported in several studies, but intraindividual heterogeneity is limited to one or two clonal types. Further studies are needed to analyse a sufficient number of isolates per patient to firmly establish whether or not a patient is truly infected with one predominant clone.

It seems that a relationship between a specific genotype of *P. gingivalis* and the periodontal status of the host has not yet been established. This subject has importance in understanding the virulence mechanisms of *P. gingivalis* and to answer the question whether *P. gingivalis* is a member of the indigenous oral flora thereby behaving as an opportunistic pathogen or if it is exogenous and thus an obligate pathogen.

Acknowledgement.—This study was supported by a grant to Nurdan Özmeriç from the Norwegian Research Council for Science and the Humanities.

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Received for publication 7 March 2000
Accepted 18 April 2000