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Phenotypes, serotypes and antibiotic susceptibility of Swedish *Porphyromonas gingivalis* isolates from periodontitis and periodontal abscesses

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This study was conducted to reveal phenotypic, serological subtypes and antibiotic susceptibility among fresh isolates of *Porphyromonas gingivalis* in a Swedish population with periodontitis and periodontal abscess. Fifty-five subgingival strains were isolated and tentatively designated as *P. gingivalis* from 55 consecutive paper-point samples taken from 51 patients with periodontitis (at least one site with >6-mm pocket depth) in Sweden and were sent in for microbiological evaluation. Eight *P. gingivalis* strains from periodontal abscesses were also included. Four *P. gingivalis* strains served as reference and another four type strains were included. The strains were characterized by colony morphology, biochemical tests, enzyme profile, gas–liquid chromatography and antibiotic susceptibility. The strains were further characterized for whole cell protein profiles using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and were identified to serotype by specific monoclonal antibodies. Among the 55 *P. gingivalis* strains 35 had smooth (S), 13 rough (R) and seven semi-rough colony morphologies. All strains were phenotypically homogeneous in biochemical tests, enzyme profile and antibiotic susceptibility. All strains produced phenylacetic acid and α-fucosidase. Almost all (96%) of the subgingival strains, but relatively fewer (62%) of the abscess strains, belonged to serotype A. Two subgingival and three abscess strains were classified as serotype B. No specific SDS–PAGE protein profiles were recorded for the two serotypes. The *P. gingivalis* strains from Swedish periodontitis cases showed homogeneity in terms of biochemical phenotypes and antibiotic susceptibility patterns. The strains fell into two serotypes, of which serotype A predominated in the periodontitis cases and serotype B was overrepresented in periodontal abscesses.

Key words: antibiotic susceptibility; periodontitis; phenotype; *Porphyromonas gingivalis*; serotype

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Porphyromonas gingivalis is an asaccharolytic gram-negative anaerobic rod that forms greenish-black pigmented colonies on blood agar plates. It is distinguished from other pigmented gram-negative rods, e.g. *Prevotella* species, by its haemagglutinating activity and lack of auto-fluorescence in UV-light (34, 35). It is further characterized and differentiated from other

Porphyromonas species by its production of phenylacetic acid. *P. gingivalis* has been strongly associated with deep periodontal pockets and considered a potential cause of periodontal disease progression (3, 15, 37). It expresses a number of unique potential virulence factors, e.g. fimbriae, capsule, proteinases and other enzymes with a potential impact on its pathogenicity (16).

Considerable heterogeneity among isolates, including variable pathogenicity in experimental animal models has been noticed (4, 10, 11, 28, 38). One group of strains, represented by the type strain ATCC 33277, led to abscesses with a rather local appearance, while another group, represented by the strains W50 and W83, formed spreading abscesses which often caused the death of the animals by sepsis. It is not known with what frequency these two pathogenic groups occur among periodontitis patients and to what extent the observed differences in virulence correlate with the disease severity. This is partly explained by the lack of phenotypic markers that could disclose these virulence subtypes.

Attempts have been made to correlate the pathogenicity in animal test systems with the expression of various surface antigens. Laine et al. (18) showed that *P. gingivalis* could be classified into six novel serotypes based on different capsular polysaccharide antigens (serotype K1–6). The presence of non-K typable strains were common, however the presence/absence of a capsule could not fully explain the difference in pathogenicity (19, 21). Parent et al. (32) and Naito et al. (27) demonstrated antigenic diversity of *P. gingivalis*, thereby confirming the results of Laliberte and Mayrand (22). By using cross-immunoelectrophoresis with polyclonal anti-*P. gingivalis* antibodies, it was possible to recognize two groups of isolates, of which one had a comparatively higher pathogenic potential. The less virulent group included the reference strain ATCC 33277 and harboured mainly strains isolated from healthy patients. These results were further substantiated by Gmür et al. (9) using monoclonal antibodies (mAbs) which grouped *P. gingivalis* into two serotypes whereby one group (serotype A) again included the less virulent strain ATCC 33277 and the other group (serotype B) contained the two related and more virulent strains W50 and W83. So far, serotype B has been found only infrequently in clinical material (8) and the prevalence of this *P. gingivalis* subtype in periodontally diseased patients is not known.

This study was conducted to reveal phenotypic subtypes, mAb-defined serotypes and antibiotic susceptibility among fresh subgingival isolates of *P. gingivalis* from patients of a homogeneous Swedish Caucasian population with chronic periodontitis or acute periodontal abscesses.

Material and methods

Bacterial strains

Fifty-five *P. gingivalis* strains (labelled PgS 1–55) were isolated from 55 positive consecutive samples sent in from periodontal specialist clinics in the west and southern part of Sweden for microbiological evaluation in the Laboratory of Oral Microbiology at Göteborg University. The samples were taken from 52 Swedish Caucasian patients with at least one site exhibiting >6-mm pocket depth. In four patients the *P. gingivalis* strains were isolated from two different deep pockets of the same patient. The patients had undergone their initial treatment with mechanical debridement, professional tooth cleaning and hygiene instruction, however the sampling sites were regarded as 'refractory' to the initial treatment. The patients had taken no antibiotics in the 6 months before the sampling occasion. Additionally, eight *P. gingivalis* strains (labelled PgA 1–8) were isolated from periodontal abscesses (13). Furthermore, two strains of *P. gingivalis*, OMGS 769 and OMGS 788, isolated from Kenyans (5), and one isolate (OMGS 984) from a mucosal scraping (dorsum of the tongue) from a patient without periodontitis, and one strain (OMGS 673) isolated from an infected necrotic root canal were included as reference strains. Altogether 67 own isolates of *P. gingivalis* were used for the phenotypical characterization.

P. gingivalis FDC 381 or ATCC 33277 and W83 or W50 were used as type strains. Moreover, *Porphyromonas endodontalis* (OMGS 926; formerly B11a-e from G. Sundquist, Umeå, Sweden) and one strain of *Prevotella intermedia* (OMGS 969) were included.

Isolation and preliminary identification to species level

The subgingival samples were collected with three paper points inserted simultaneously into the same periodontal pocket and left in place for 15 s. The paper points were removed at the same time, immediately transferred into transport medium VMGA III (Viability Medium Göteborg Anaerobic III according to Möller, 24) and processed in the laboratory within 24 h. The samples were thoroughly mixed and serially diluted to 10^{-4} using VMG I (Viability Medium Göteborg I according to Möller, 24). Then, 0.1 ml of each dilution was uniformly distributed onto Brucella agar plates (BBL, Microbiology Systems Cockeysville, MD) enriched with 5% defibrinated horse blood, 0.5% haemolysed horse blood and 5 mg/ml menadione.

The plates were analysed after 7–9 days of anaerobic incubation in jars using the hydrogen combustion method (25). Representative pigmented colonies were subjected to Gram-stain procedures; moreover, red auto-fluorescence under UV light (360 nm) and agglutination of red blood cells were evaluated by titration (34).

P. gingivalis was tentatively identified as gram-negative anaerobic rods forming pigmented colonies, with the ability to agglutinate red cells and without autofluorescence at 360 nm. Based on colony morphology at primary isolation and at subcultivation, the following colony morphotypes were distinguished: S = smooth, R = rough or SR = semi-rough (intermediate variants).

Gas–liquid chromatography

Bacterial metabolic products formed in peptone yeast medium (Bacto Yeast Extract, Becton Dickinson and Company, Sparks, MD) with 1% glucose were evaluated using a gas–liquid chromatograph with a flame ionization detector (Sigma 2B, Perkin-Elmer, Norwalk, CN) as outlined in the Virginia Polytechnic Institute manual (14). The glass column of the chromatograph was packed with 5% AT 1000 (Altech Associates, Deerfield, IL) on chromosorb GHP 100/120 mesh (Johns-Manville, Denver, CO). The carrier gas was nitrogen (30 ml/min), the injection port temperature was 150°C and the oven temperature was 120°C. One microlitre of the ether-extracted or methylated samples was used, and the results were compared with standard solutions of volatile fatty acids (14).

Biochemical tests

The peptone-yeast medium broth (Becton Dickinson) was used as the basal medium for analyses of fermentation of carbohydrates and derivates by *P. gingivalis* strains (14). The preparation and inoculation of fermentation tubes were carried out according to the Virginia Polytechnic Institute manual (14).

Enzymatic profiles

The API-ZYM colorimetric kit system (API System, Bio-Merieux, Marcy l'Etoile, France) for detection of enzymes was used with all strains according to the manufacturer's directions. Colour reactions were read from grade 0 to 5, whereby 0 indicates no enzyme activity, 1 and 2 indicate weak activity, and 3–5 indicate strong, significant enzyme activity.

Antibiotic susceptibility

Both the disc-diffusion (for primary screening) and the agar plate dilution [for minimum inhibitory concentration (MIC) determination] methods (14) were used for antibiotic susceptibility testing of the 59 periodontitis and reference strains. Susceptibility was tested to the following antibiotics: penicillin-G, isoxapenicillin, ampicillin, tetracycline, erythromycin, metronidazole, clindamycin, oxytetracycline, kanamycin and tinidazole, all obtained from Sigma (St Louis, MO). The eight strains isolated from periodontal abscess were tested against penicillin-G, isoxapenicillin, ampicillin, tetracycline, erythromycin, metronidazole and clindamycin using the E-test (Biodisk, Solna, Sweden).

SDS–PAGE whole protein profiling

Sodium dodecyl sulphate–polyacryamide gel electrophoresis (SDS–PAGE) was performed in a mini-protean unit (Bio-Rad Laboratories, Sundbyberg, Sweden) at 200 V for 45 min by using a vertical 0.75-mm-thick slab gel containing 7.5% (weight/weight) polyacrylamide. Bacterial samples were prepared by whole-cell sonication at 50 W for 1 min. Extracts were then boiled in sample buffer (17) for 5 min. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250; myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine plasma albumin (66 kDa), ovalbumin (45 kDa) served as high range marker proteins (Bio-Rad standard).

Subtyping with mAbs

Hybridoma culture supernatants containing mAbs 50BG2.1, 60BG1.3 or 48BG1.1 with specificity for *P. gingivalis* (9) were used to subtype the subgingival and abscess isolates. The strains FDC381 and W83 were used as references for serotypes A and B, respectively (9). Subtyping was performed by indirect immunofluorescence as described previously (40). Serotype B was defined by a positive reaction with mAb 50BG2.1, while serotype A strains reacted with 60BG1.3 or 48BG1.1 only.

Results

Colony morphology

On primary isolation of *P. gingivalis*, colony pigmentation over 7 days of incubation varied among the 55 subgingival strains from yellowish-green to black. The majority of colonies were greenish-black. Ten of the 55 subgingival *P. gingivalis* strains were identified as rough (R) and were described also as strongly adherent to the agar surface. Additionally, three strains could be described as semi-rough (SR). There was no relation between the R and SR growth patterns and pigmentation. All eight strains from abscesses had smooth colony morphology.

At isolation, 45 strains were selected based on the absence of auto-fluorescence under long-wave UV-light and 22 were selected because of a positive haemagglutination reaction. When these procedures were applied to all isolated strains and two type strains of *P. gingivalis* (FDC381 and W83), all strains were auto-fluorescence negative and red cell agglutination positive (Table 1). However, 12 strains (six of them R-strains and the type strain W83) showed a slightly weaker agglutination activity (<1/1000) than the rest. *P. endodontalis* OMGS 926 showed weak (1/16) agglutination activity and *P. intermedia* OMGS 969 showed no agglutination activity; both strains showed red-orange auto-fluorescence.

Biochemical reactions

All *P. gingivalis* strains, including the type strains, were negative for nitrate reduction, showed a positive reaction for indole and gelatinase and most strains could proteolyse milk (Table 2). All isolates, except the *P. endodontalis* and *P. intermedia* reference strains, produced phenyl acetic acid.

API-ZYM

The results of the enzyme profile on the API-ZYM strip of the 55 subgingival and eight abscess strains are shown in Table 2. All *P. gingivalis* strains showed positive alkaline and acid phosphatase and all revealed a positive trypsin and *N*-acetyl-β-glucosaminidase reaction. Other enzyme activities were weak or absent. Both *P. endodontalis* and *P. intermedia* were negative for trypsin and *N*-acetyl-β-glucosaminidase.

Antibiotic susceptibility tests

All strains of *P. gingivalis* were sensitive and gave wide inhibition zones upon exposure to penicillin G, tetracycline, ampicillin, clindamycin, metronidazole, tinidazole and erythromycin using the antibiotic disc method.

The susceptibilities of the strains for antibiotics by the agar dilution method are presented in Fig. 1. Generally, all *P. gingi-*

Table 1. Biochemical testing of 67 *Porphyromonas gingivalis* strains (55 strains from periodontitis, eight strains from abscess, two type strains), one *Porphyromonas endodontalis* and one *Prevotella intermedia* strain

	P. gingivalis (periodontitis)			*P. gingivalis*				
					Type strains			
Biochemical	S-strains (42)	SR-strains (3)	R-strains (10)	Abscess (8)	W83	381	*P. endod.* (OMGS926)	*P. int.* (OMGS969)
UV-fluorescence	–	–	–	–	–	–	+	+
Agglutination of sheep red blood cells	+ (5 weak)[1]	+ (1)[1]	+ (6)[1]	+	+	+	Weak (+)	–
Fermentation of glucose	–	–	–	–	–	–	–	+
Nitrate of nitrate	–	–	–	–	–	–	–	+
Indole	+	+	+	+	+	+	–	–
Milk proteolysis	+ (10[1])	+	+ (1)[1]	+	+	+	–	–
Gelatinase	+	+	+	+	+	+	+	Weak (+)
Phenyl acetic acid production	+	+	+	+	+	+	–	–

[1]Number of strains with weak reaction in brackets.
P. endod.; *Porphyromonas endodontalis*; *P. int.*, *Prevotella intermedia*.

Table 2. Enzymatic characterization of 67 *Porphyromonas gingivalis* strains (55 subgingival isolates, eight strains from abscess and two type strains), one *Porphyromonas endodontalis* and one *Prevotella intermedia* strain by the API-ZYM test

	P. gingivalis (periodontitis)			*P. gingivalis*			
Enzyme reaction	Strong (3, 4, 5)	Weak (1, 3)	No (0)	Abscess (8)	Type strains (W83, 381)	*P. endod.* (1)	*P. int.* (1)
Alkaline phosphatase	57	2	0	+	+[a]	+	+
Butyrate	0	48	11	w	w	w	–
Caprylate	0	47	12	w	w	–	–
Myristase	0	0	59	–	–	–	–
Leucine aminopeptidase	0	1	58	–	–	–	–
Valine aminopeptidase	0	0	59	–	–	–	–
Cystein aminopeptidase	0	0	59	–	–	–	–
Trypsin	57	2	–	+	+	–	–
Chymotrypsin	0	1	58	–	–	–	–
Acid phosphatase	50	9	0	+	+	+	+
Phosphoaminidase	0	38	21	w	w	w	–
α-Galactosidase	0	0	59	–	–	–	–
β-Galactosidase	0	2	57	–	–	–	–
β-Glucuronidase	0	0	59	–	–	–	–
α-Glucosidase	0	1	58	–	–	–	–
N-acetyl-α-glucose aminidase	53	6	0	+	+	–	–
α-Mannosidase	0	1	58	–	–	–	–
α-Fucosidase	0	1	58	–	–	–	–

'+' denotes strong activity corresponding to 3, 4 and 5; 'w' denotes (1, 2) and '–' denotes no activity (0).
P. endod.; *Porphyromonas endodontalis*; *P. int.*, *Prevotella intermedia*.

valis strains tested showed an overall susceptibility to all tested antibiotics except for kanamycin, for which all strains showed a susceptibility of 100 μg/ml or more (Fig. 2). One strain showed reduced sensitivity for penicillin G (MIC 1 μg/ml), four strains showed reduced sensitivity for ampicillin (MIC >0.5 μg/ml) and nine strains showed reduced sensitivity for clindamycin (MIC >0.1 μg/ml), which differed with at least one dilution step from the majority of strains. However, none of these strains were considered resistant to those antibiotics using a cut-off point of 4 μg/ml. The *P. intermedia* strain OMGS 969 was sensitive to kanamycin (MIC 0.5 μg/ml).

SDS–PAGE protein profiling

The 55 subgingival *P. gingivalis* isolates and the type strains FDC 381 and W83 were divided into six major protein groups (Groups Ia to IId) based on protein banding patterns (Fig. 3). Most of strains belonged to Groups Ia and IIa respectively. The SDS–PAGE groups corresponded neither to colony morphology groups (S, R or SR groups) nor to the serotype classification (see below).

Serotype classification using mAbs

All strains reacted positively with mAbs 60BG1.3 and 48BG1.1, including *P. gingivalis* reference strains FDC381 and W83. Three strains W83, PgS 3 (OMGS 789), and PgS 10 (OMGS 719) reacted positively with 50BG2.1, indicating their identity as serotype B (Table 3). Among the eight abscess strains, three strains (37%) were identified as serotype B.

Discussion

This study has confirmed a considerable homogeneity in phenotypic characteristics and antibiotic susceptibility among *P. gingivalis* isolates, allowing easy differentiation from other black-pigmented species. The colony morphology can differ from smooth to rough but subtypes could not otherwise be distinguished phenotypically or by their SDS–PAGE banding pattern. The *P. gingivalis* isolates were mainly classified as serotype A; only two of 55 (4%) Swedish periodontitis isolates and three of eight (38%) abscess isolates fell into serotype B.

The 55 *P. gingivalis* isolates from Swedish periodontitis cases showed a strong homogeneity in the biochemical tests. Thus, they were all haemagglutinating, produced phenylacetic acid and were positive for trypsin-like activity in the API-ZYM test (33). None showed red/orange fluorescence in UV-light. The *P. intermedia* and *P. endodontalis* strains were easily identified, which confirms that the phenotypic characterization is reliable for the identification of *P. gingivalis* to the species level. It can be concluded that *P. gingivalis* easily distinguished from other black-pigmented species based on the phenotypical characteristics, however no biochemical subgroups were disclosed.

The antibiotic susceptibility patterns of the *P. gingivalis* strains also confirmed previous reports (29, 31). Antibiotics have commonly been used by dental practitioners in Sweden as an adjunct to non-surgical and surgical periodontal treatment during the last couple of decades. Tetracycline was used initially and later amoxicillin/ampicillin and metronidazole (often in combination) were used as the drugs of choice (2, 36, 41). Applebaum et al. (1) have reported that antibiotic resistance has been developed against anaerobic microorganisms (e.g. *Prevotella* spp. and *Fusobacterium* spp.). A higher frequency of resistance in subgingival anaerobic bacteria has been reported in Spain than in the Netherlands with its more restricted antibiotic prescriptions (39). However, in neither of these two countries were resistant strains of *P. gingivalis* found against the most commonly prescribed antibiotics in periodontal treatment. Although it was not possible to obtain reliable information on the antibiotic intake during the past 20 years among the patients in the present study, no resistance was found among the *P. gingivalis* isolates against those antibiotics commonly used in the treatment of periodontal disease (penicillin, amoxicillin, tetracycline, clindamycin and metronidazole). So far, plasmids in *P. gingivalis* against these antibiotics have not been reported (30). It can be concluded that the ability for *P. gingivalis* to develop antibiotic resistance is limited.

Many gram-negative rod-shaped bacteria can grow in smooth and rough colony forms on agar plates. The smooth form has been related to the length of the polysaccharide side chain of lipopolysaccharide (O-antigen) or the presence and thickness

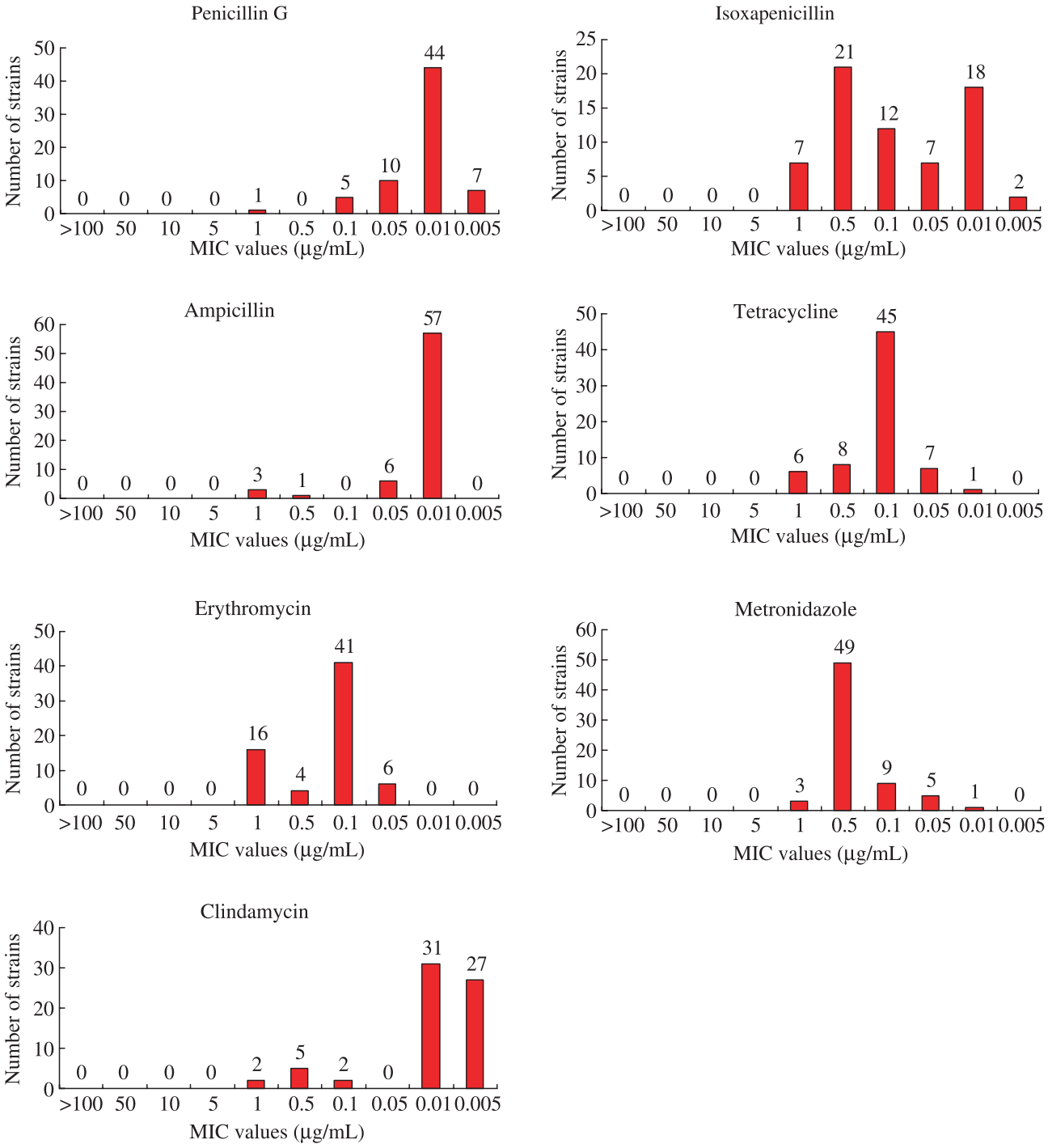

Fig. 1. Antibiotic susceptibility of 67 *Porphyromonas gingivalis* strains tested with the agar dilution method and E-test.

of a capsule. Consequently, the rough forms have a shorter polysaccharide chain or a capsule that is thinner or absent. A variation in virulence may therefore exist between the two morphotypes. Laine et al. (19) have reported on the serological classification of *P. gingivalis* using K antigens; they found that although many strains could be typed, a substantial number were K-antigen non-typable, indicating the presence of no or a very thin capsule. R forms of *P. gingivalis* are not K-antigen typable (20). The R-strains in the present study were all mAbs serogroup A strains and did not show any distinctive pattern in SDS–PAGE. It was not possible to conclude whether the R-forms were antigenically different from the S-forms and it is not possible at this stage to explain the appearance of rough colony morphology in *P. gingivalis*.

In the serological classification of *P. gingivalis* antigens other than the K-antigens have been used, however the serology is far from clear because of variations in either the antigen expression or the methods used. Using mAbs, Gmür

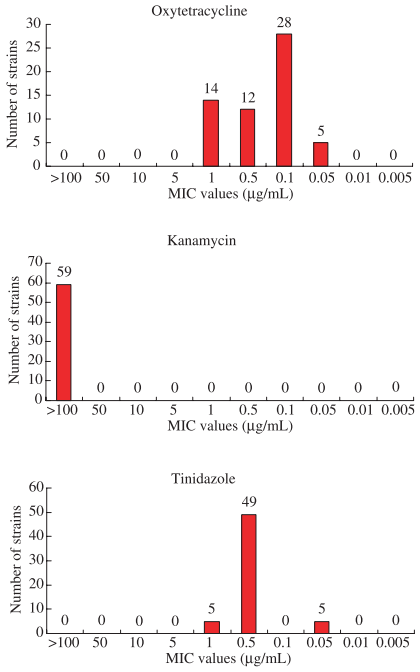

Fig. 2. Antibiotic susceptibility of 59 *Porphyromonas gingivalis* strains tested with the agar dilution method for oxytetracycline, kanamycin and tinidazole.

et al. (9) described two antigenically distinct types (A and B) that could possibly be identical to the two types reported by Fisher et al. (7). Later, a third serotype (C) was identified which seemed restricted to *P. gingivalis* isolates from Pima Indians native in Arizona, USA (7, 42). Notably, the commonly used virulent strains W50 and W83 reacted with the serotype B-specific mAb 50BG2.1, while FDC 381 and ATCC 33277 fell into serotype A. This former pair of strains has been found to be of the same genotype and eventually may have derived from the same patient and clone, although the source of the primary isolation of the two strains is unclear. The original speculation that the mAb-defined serotypes could coincide with the aforementioned virulence groups causing either spread or localized subcutaneous infections in mice, failed in the sense that certain serotype B strains (e.g. 274 from G. Sundqvist, Umeå, Sweden) produced a local infection and certain serotype A strains (e.g. B262) produced spread infection (8). Nagata et al. (26) used a different serological system to subtype *P. gingivalis* and found that enzyme activity of *P. gingivalis* was unrelated to the serological classification. However, phenotypic classification of *P. gingivalis* related to the mAb serotypes A and B failed in the present study.

In this study, only two strains of the 55 subgingival *P. gingivalis* strains fell into the serotype B, which shows that this serotype occurs only sparsely among Swedish periodontitis cases. This confirms the report by Gmür (8) who failed to detect serotype B-specific *P. gingivalis* isolates in subgingival plaque samples. Conclusively, strains of *P. gingivalis* serotype A are generally represented in cases of chronic periodontitis. We do not currently know if serotype B is more commonly found in other populations or if it is just an uncommon finding worldwide. The two Kenyan isolates both fell into serotype A.

Interestingly, serotype B was more common among the periodontal abscess isolates, although only eight strains were investigated. It should also be mentioned that one of the isolates (PgS3) of the subgingival isolates was from a diabetes patient and a site with a severe periodontal destruction all the way down to apex. This at least indicates that the serotype B in fact may include more virulent isolates sampled from more active periodontal lesions.

It was not possible in this study to find any SDS–PAGE protein banding pattern that correlated with the mAb serotypes. Ebersole and Steffen (6) demonstrated quite distinctive patterns for the three *P. gingivalis* strains (ATCC 33277, W50 and A7A1–28) representing serotypes A, B and C of Fisher et al. (7), respectively. A multiple serotype response was detected in the humoral antibody level in periodontitis cases and indicated that several cross-reacting antigens in the cell wall or outer membrane are involved in the immune response (6). It is also possible that these three type strains are not represented by a serotype SDS–PAGE protein-banding pattern in general.

In conclusion, this study has demonstrated that *P. gingivalis* strains isolated based on their colony morphology, haemagglutinating ability or level of non-fluorescence were correctly classified as *P. gingivalis*; heterogeneity in colony morphology was not related to any other phenotypical, antigen or SDS-protein banding patterns. The *P. gingivalis* strains showed a strong homogeneity in biochemical tests and susceptibility to antibiotics. The use of mAbs revealed the majority of strains to be serotype A and only two subgingival and three periodontal

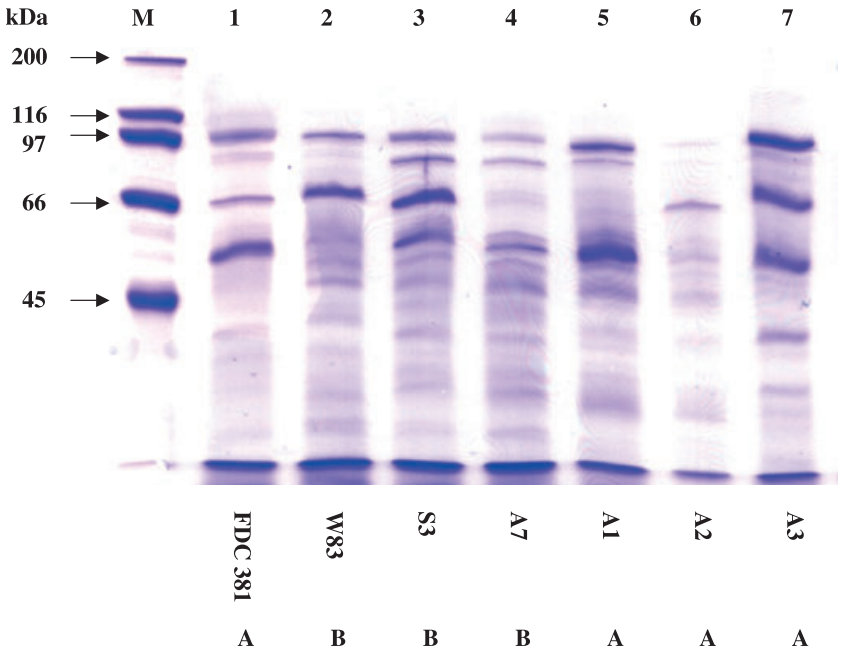

Fig. 3. SDS–PAGE protein profiles of *Porphyromonas gingivalis* strains (lanes 1–7). Lane M indicates protein markers. A and B indicate serotype.

Table 3. Identification of *Porphyromonas gingivalis* isolates with serotype specific monoclonal antibodies

Strain	Serogroup A	Serogroup B
Type strains	1 (ATCC 33277)	1 (W83)
Reference strains	4	0
Subgingival strains (periodontitis)	53	2
Abscess strains	5	3

abscess strains were serotype B. A considerable heterogeneity at the genotype level has been reported (23) and an association with some genotypes and virulence is indicated (12). Further studies on the genotypes and specific virulent biotypes are suggested for the isolates presented in this study.

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References

1. Appelbaum PC, Spangler SK, Shiman R, Jacobs MR. Susceptibilities of 540 anaerobic gram-negative bacilli to amoxicillin, amoxicillin-BRL 42715, amoxicillin-clavunalate, temafloxacin and clindamycin. Antimicrob Agents Chemother 1992: **36**: 1140–1143.
2. Asikainen S, Dahlén G, Klinge B, Olsen I, Westergaard J. Antibiotika vid parodontala behandlingar. Tandläkartidningen 2002: **94**: 26–33.
3. Bragd L, Dahlén G, Wikström M, Slots J. The capability of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis* and *Bacteroides intermedius* to indicate progressive periodontitis; a retrospective study. J Clin Periodontol 1987: **14**: 95–99.
4. Dahlén G, Slots J. Experimental infections by *Bacteroides gingivalis* in non-immunized and immunized rabbits. Oral Microbiol Immunol 1989: **4**: 6–11.
5. Dahlén G, Manji F, Baelum V, Fejerskov O. Black-pigmented *Bacteroides* species and *Actinobacillus actinomycetemcomitans* in subgingival plaque of adult Kenyans. J Clin Periodontol 1989: **16**: 305–310.
6. Ebersole JL, Steffen MJ. Human antibody responses to outer envelope antigens of *Porphyromonas gingivalis* serotypes. J Periodontal Res 1995: **30**: 1–14.
7. Fisher JG, Zambon JJ, Chen P, Genco RJ. *Bacteroides gingivalis* serogroups and correlation with virulence. J Dent Res 1986: **65**: 816 (abstract).
8. Gmür R. *Value of new serological probes for the study of putative periodontal pathogens. A survey after five years of application*. Chicago, IL: Quintessence Publishing Co, Inc., 1995. Habilitation thesis.
9. Gmür R, Werner-Felmayer G, Guggenheim B. Production and characterisation of monoclonal antibodies specific for *Bacteroides gingivalis*. Oral Microbiol Immunol 1988: **3**: 181–186.
10. Grenier D, Mayrand D. Selected characteristics of pathogenic and non-pathogenic strains of *Bacteroides gingivalis*. J Clin Microbiol 1987: **25**: 738–740.
11. Grenier D, McBride BC. Preliminary studies on the influence of in vivo growth on selected characteristics of *Porphyromonas gingivalis* W83. Microb Ecol Health Dis 1991: **4**: 105–111.
12. Griffen AL, Lyons SR, Becker MR, Moeschberger ML, Leys EJ. *Porphyromonas gingivalis* strain variability and periodontitis. J Clin Microbiol 1999: **37**: 4028–4033.
13. Hafström CA, Wikström MB, Renvert S, Dahlén GG. Effect of treatment on some periodontopathogens and their antibody levels in periodontal abscesses. J Periodontol 1994: **65**: 1022–1028.
14. Holdeman LV, Cato EP, Moore WE. *Anaerobic laboratory manual*. Blacksburg, VA: Virginia Polytechnic Institute and State University, 1975.
15. Holt SC, Ebersole JL, Felton J, Brunsvold M, Kornman KS. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of pertiodontitis. Science 1988: **239**: 55–57.
16. Holt SC, Kesavalu L, Walker S, Genco CA. Virulence factors of *Porphyromonas gingivalis*. Periodontol 2000 1999: **20**: 168–238.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970: **227**: 680–685.
18. Laine ML, Appelmelk BJ, van Winkelhoff AJ. Novel polysaccharide capsular serotypes in *Porphyromonas gingivalis*. J Periodontal Res 1996: **31**: 278–284.
19. Laine ML, Appelmelk BJ, van Winkelhoff AJ. Prevalence and distribution of six capsular serotypes of *Porphyromonas gingivalis* in periodontitis patients. J Dent Res 1997: **76**: 1840–1844.
20. Laine ML. Periodontal disease: virulence factors of *Porphyromonas gingivalis* and polymorphisms in the host IL-1 gene family. Thesis, Amsterdam: ACTA, 2000.
21. Laine ML, van Winkelhoff AJ. Virulence of six capsular serotypes of *Porphyromonas gingivalis* in a mouse model. Oral Microbiol Immunol 1998: **13**: 322–325.
22. Laliberté M, Mayrand D. Characterisation of black-pigmented *Bacteroides* strains isolated from animals. J Appl Microbiol 1983: **55**: 247–252.
23. Loos BG, Mayrand D, Genco RJ, Dickinson DP. Genetic heterogeneity of *Porphyromonas* (*Bacteroides*) *gingivalis* by genomic fingerprinting. J Dent Res 1990: **69**: 1488–1493.
24. Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr 1966: **74** (suppl): 1–380.
25. Möller O, Möller ÅJR. Some methodological considerations for anaerobic cultivation. Acta Pathol Microbiol Scand 1961: **51**: 245–247.
26. Nagata A, Manyoshi T, Sato M, Nakamura R. Serological studies of *Porphyromonas gingivalis* and correlation with enzyme activities. J Periodontal Res 1991: **26**: 184–190.
27. Naito Y, Okuda K, Kato T, Takazoe I. Monoclonal antibodies against surface antigens of *Bacteroides gingivalis*. Infect Immun 1985: **50**: 231–235.
28. Neiders ME, Chen PB, Suido H et al. Heterogeneity of virulence among strains of *Bacteroides gingivalis*. J Periodontal Res 1989: **24**: 192–198.
29. Notten FJW, Nieman FHM, Mikx FHM. Antibiotype of *Bacteroides gingivalis* assessed by antimicrobial disks and multivariate analysis. J Clin Microbiol 1985: **22**: 1020–1024.
30. Olsen I, Shah HN, Gharbia SE. Taxonomy and biochemical characteristics of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. Periodontol 2000 1999: **20**: 14–52.
31. Pajukanta R, Asikaianen S, Forsblom B, Piekkola M, Jousimies-Somer H. Evaluation of the E test for antimicrobial susceptibility testing of *Porphyromonas gingivalis*. Oral Microbiol Immunol 1994: **9**: 123–125.
32. Parent R, Mouton C, Lamonde L, Bouchard D. Human and animal serotypes of *Bacteroides gingivalis* defined by crossed immunoelectrophoresis. Infect Immun 1986: **51**: 909–918.
33. Slots J. Enzymatic characterization of some oral and nonoral gram-negative bacteria with the API ZYM system. J Clin Microbiol 1981: **14**: 288–294.
34. Slots J, Genco RJ. Direct hemagglutination technique for differentiating *Bacteroides asaccharolyticus* oral strains from nonoral strains. J Clin Microbiol 1979: **10**: 371–373.
35. Slots J, Reynolds HS. Long-wave UV light fluorescence for identification of black-pigmented *Bacteroides* spp. J Clin Microbiol 1982: **16**: 1148–1151.
36. Slots J, Rosling BG. Suppression of the periodontopathic microflora in localized juvenile periodontitis by systemic tetracycline. J Clin Periodontol 1983: **10**: 465–486.
37. Socransky SS, Smith C, Haffajee AD. Subgingival profiles in refractory periodontal disease. J Clin Periodontol 2002: **29**: 260–268.
38. Van Steenbergen TJM, Delemarre FGA, Namavar F, de Graaff J. Difference virulence within the species *Bacteroides gingivalis*. Antonie van Leeuwenhoek 1987: **53**: 233–244.
39. van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in the Netherlands and Spain. J Clin Periodontol 2005: **32**: 893–898.
40. Werner-Felmayer G, Guggenheim B, Gmür R. Production and characterization of monoclonal antibodies against *Bacteroides forsythus* and *Wolinella recta*. J Dent Res 1988: **67**: 548–553.
41. Winkel EG, van der Weijden GA, van Winkelhoff AJ, Timmerman MF, van der Velden U. Metronidazole plus amoxicillin in the treatment of adult periodontitis patients. A double-blind placebo-controlled study. J Clin Periodontol 2001: **28**: 296–305.
42. Zambon JJ, Reynolds HS, Fisher JG, Shlossman M, Dunford R, Genco RJ. Microbiological and immunological studies of adult periodontitis in patients with non-insulin-dependent diabetes mellitus. J Periodontol 1988: **59**: 23–31.