

Porphyromonas gingivalis ribotyping

1 . 2 . 1 . 1 . 1 . 1

,

2

I.

P. gingivalis 가
15-20) fimbriae

. Fimbriae 가 21,22)

fimbrillin ,

fimbriae

가 23).

, 가
P. gingivalis 24-29).

가

P. gingivalis

500
15 20

1).

Porphyromonas gingi -

valis

30

P. gingivalis

2,3),

P. gingivalis

P. gingi -

4-9). P. valis

10-12)

(phenotypic method)

(genotypic method)

Biotyping³⁰⁾, serotyping³¹⁾, antibiotyping³²⁾

SDS - PAGE protein profile^{33,34)}

13,14).

gingivalis

가 rRNA) 가
16S rRNA

P. gingivalis

ribotyping

, multilocus enzyme elec-
trophoresis ribotyping P. gingivalis , ,
P. gingivalis P. gingivalis

P. gingivalis 35-37), P. gingivalis vaccine
가 가
P. gingivalis 42) 가 P.
P. gingivalis gingivalis 가

38-41), Loos
DNA P. gingivalis
(restriction fragment length polymorphism:
RFLP) ribotype 가
P. gingivalis

33
P. gingivalis 29가 DNA 16S rRNA
RFLP 40), Van ribotyping
Steenbergen P. gingivalis가 ribotype fimbriillin

가 ,
가 P. gingivalis
DNA 가 II.

DNA
41), Loos Dyer가 P. gin - 1 .
givalis 100 multilocus Y
enzyme electrophoresis 10
78가 5 22
39),
P. gingivalis 36 .
band ,

. Ribotyping
band

ribotyping 2.
16S ribosomal RNA(16S

(1) 가 6mm 3
 paper point 10
 1M reduced transport medium⁴³
 vial

(2) 10
 5% , hemin(10 μ g/ μ l),
 vitamin K₃(5 μ g/ μ l)
 BHI(Brain heart infusion, Difco. Detroit,
 MI.,USA)
 (5% CO₂, 10% H₂, 85% N₂) 10
 P. gingivalis
 BHI
 500 μ l BHI
 2 - 3

5M BHI
 (2M) glycerol(15%)
 가 - 70 , (500
 μ l) sodium dodesyl sulfate - polyacry -
 lamide , 2.5 M DNA

(3) DNA
 P. gingivalis (2.5M) 5,000g
 10
 2 (500mM
 Tris - HCl, pH 9.0, 20mM EDTA, 10mM
 NaCl, 1% sodium dodesyl sulfate)
 proteinase K(200 μ g/Ml)
 가 1 37 . DNA
 phenol, phenol/chloroform(1:1), chlo -

roform ethanol
 . DNA 70% ethanol
 200M TE (10mM Tris - HCl,
 pH 8.0, 1mM EDTA) . RNA
 RNase(50 μ g/Ml) 가 37
 30 DNA 260
 nm

(4) (Dot - blot hybridiza -
 tion)⁴⁴
 가 P. gingivalis
 DNA 16S rRNA
 P. gingivalis DNA 95
 10 2 μ l
 (254nm, 3) DNA
 digoxigenin P. gingivalis
 16S rRNA
 (5 TAC TCG TAT CGC CCG TTA TTC
 3')

Digoxigenin (DIG) oligonucleotide 3' - end
 labeling kitTM (Boehringer Mannheim,
 Germany) DIG - ddUTP
 . 20 μ l (X μ l 100 pmol
 oligonucleotide, 4 μ l CoCl₂, 1 μ l DIG -
 ddUTP, 1 μ l terminal transferase) 37
 15 2 μ l stock
 solution(1 μ l glycogen 200 μ l 0.2 mM
 EDTA) 가 .
 2.5 μ l 4 M LiCl 75 μ l ethanol
 - 20

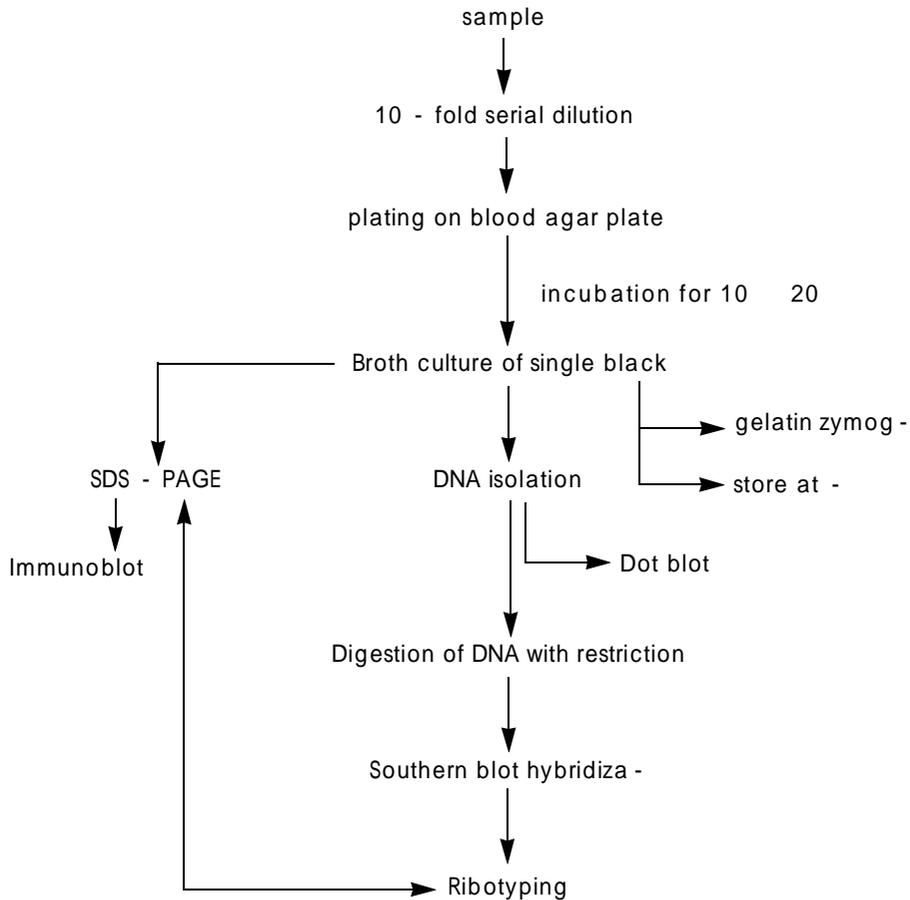
* CSPD

(Disodium 3 - (4 - methoxyspiro{1,2 - dioxetane - 3,2' - (5' - chloro)tricyclo[3.3.1.1^{3,7}]decan} - 4 - yl)phenyl
 phosphate, Boehringer Mannheim.Germany) 1:100

12,000g
 500 μ l 70% ethanol
 20
 μ l dH₂O
 (5 x SSC, 1% , 0.1% N - lau -
 roylsarkosine, 0.02% SDS) 30
 56 prehybridization
 digoxigenin
 (50 pmol, 10 μ l) 1
 I (5 x SSC,
 0.01% SDS) 15
 DIG - lumi -
 nescent detection kit (Boehringer
 Mannheim, Germany) alkaline
 phosphatase (AP)가 anti - digoxigenin
 chemiluminescence
 AP가 anti - digoxi -
 genin II (0.1%
 maleic acid, 0.15M NaCl, 0.3% Tween 20,
 pH 7.5) 1 - 2M
 CSPD * (Boehringer Mannheim,
 Germany) 5
 Whatman 3MM paper
 가
 . membrane 37 5 - 15
 X - ray
 (5) DNA Southern
 blot
 P. gingivalis ribotype
 DNA agarose
 southern - blot
 16S rRNA
 DNA KpnI (MBI Fermentas
 St. Leon - Rot, Germany) PstI (MBI
 Fermentas St. Leon - Rot, Germany) 1 μ l
 10 x buffer 1 μ l, 5 8 μ l DNA
 10 μ l가 dH₂O

37 1
 DNA 0.8% agarose gel
 . Agarose gel 0.25 N HCl 20
 , DNA agarose
 gel DNA (1.5 M NaCl, 0.5 M
 NaOH) 30 . Agarose
 DNA 20 x SSC (3 M NaCl, 0.3 M
 Sodium citrate, pH 7.0)
 gel
 2 x SSC 15
 (254 nm, 3) DNA
 (5 x
 SSC, 1% , 0.1% N - lauroyl -
 sarkosine, 0.02% SDS)
 56 30 digoxi -
 genin
 가 56 1
 I 56 15 2
 1%
 AP - conjugated anti - digoxi -
 genin 1
 AP CSPD
 (Boehringer Mannheim, Germany) 가
 X - ray film
 (6) SDS - polyacrylamide gel (SDS -
 PAGE)³⁴⁾
 ribotype
 SDS - PAGE
 (13,000g, 10)
 (50mM
 Tris - HCl, pH 6.8, 100mM dithiothreitol, 2%
 SDS, 0.1% bromophenol blue, 10% glyc -
 erol) 95 5 가 10%
 SDS - polyacrylamide gel
 . Gel 0.25% Coomassie brilliant blue
 R250 (isopropyl
 alcohol/ glacial acetic acid)

The scheme of experimental pro -



horseradish peroxidase(HRP)가 goat anti - rabbit IgG ECL western blotting detection reagents (Amersham Pharmacia Biotech., Piscataway, NJ, USA)

(7) Immunoblot ribotype fimbriae fimbrellin immunoblot SDS - PAGE semi - dryer blotter 10% skim milk가 0.1% Tween P. gingivalis strain 2561 anti - fimbrillin antibody (Ab) . 2

(8) Gelatin zymography P. gingivalis gelatin zymography 15μℓ 4μℓ (2.5% SDS, 3% sucrose, 0.005% bromophenol blue) 0.2% gelatin SDS -

Table 1. Porphyromonas gingivalis isolates from rapidly progressive periodontitis patients and ribo - types

Patient no.(ID)	Sex & Age	No. of isolates (Designation of isolates)	Ribotype	
			KpnI	PstI
1(2 - 46)	female 28	1(A1)	III	c
2(10 - 36)	male 36	8(B1 - 8)	I	e
3(14 - 16)	female 29	2(C1 - 2)	IV	c
4(15 - 21)	male 25	2(D1 - 2)	V	d
5(17 - 26)	male 26	1(E1)	I	f
		1(E2)	I	g
6(19 - 11)	female 22	4(F1 - 4)	I	e
7(21 - 11)	male 30	3(G1 - 3)	VI	a
		2(H1 - 2)	II	b
8(26 - 11)	male 30	8(I1 - 8)	II	b
9(4 - 43)	female 32	1(J1)	I	e
10(27 - 16)	female 31	3(K1 - 3)	I	e

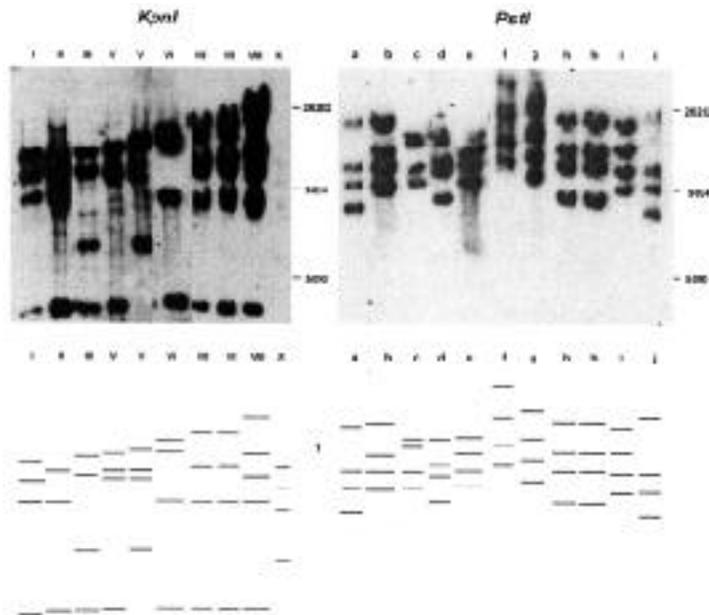


Figure 1. Ribotype patterns of representative *P. gingivalis* strains isolated from RPP patients and 4 reference strains. DNA of the isolates was digested with restriction enzymes Kpn I and Pst I, respectively and probed with a 16S rRNA - specific oligonucleotide probe for *P. gingivalis*. Reference strains of *P. gingivalis* were included: ATCC 33277(ribotype /h); strain 2561(ATCC 33277, ribotype /h); strain A7A1 - 28(ribotype /i), strain W50(ATCC 53978,

polyacrylamide gel(8%)
 . Gel (2.5% Triton X - 100
 50 mM Tris - HCl, pH 7.5) 30 2
 SDS . Gel 37
 (0.15 M NaCl, 10 mM CaCl₂,
 50 mM Tris - HCl, pH 7.5) 18
 Coomassie Blue R -
 250(0.05%, isoprophyl alcohol: glacial
 acetic acid: dH₂O =1:1:8) iso -
 prophyl alcohol: glacial acetic acid:

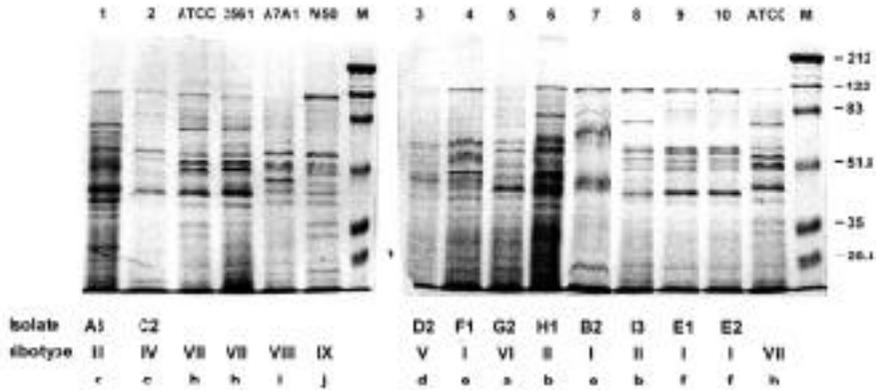


Figure 2. Coomassie blue - stained SDS - PAGE separation patterns of whole cell sonicates(10 - 20µg) of representative *P. gingivalis* isolates using 10% polycarylamide gel. Reference strains of *P. gingivalis* were included: ATCC(ATCC 33277), 2561(ATCC 33277), A7A1 - 28, W50(ATCC

dH₂O(1:1:8)

oligonucleotide

KpnI

PstI

DNA

III.

1. *P. gingivalis*

ribotype

ribotype

KpnI

6, PstI

7

(Figure 1),

ribotype

36 *P. gingivalis*

P.

ATCC

gingivalis

KpnI

10

5

16S rRNA

I

2

II,

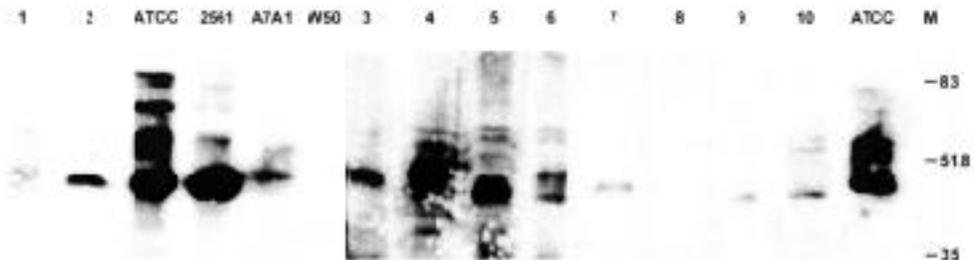


Figure 3. Immunoblot of whole cell sonicates of *P. gingivalis* isolates with polyclonal antibodies directed against fimbrillin of *P. gingivalis* 2561. After whole cell extracts(10 - 20µg) of *P. gingivalis* isolates was separated on 10% SDS - PAGE, proteins were transferred to nitrocellulose membranes and incubated with anti - fimbrillin polyclonal antibodies directed against *P. gingivalis* 2561. Goat anti - rabbit IgG labelled horseradish peroxidase(HRP) was used as the second antibody. Detection was performed using ECL immunodetection procedure. The same isolates were included as in Fig. 2. Reference strains of *P. gingivalis* were includ -

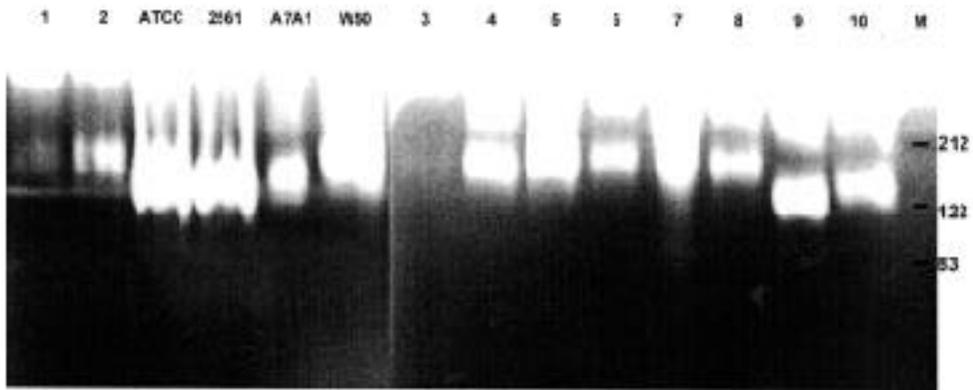


Figure 4. Zymograms of whole cell sonicates of *P. gingivalis* isolates. After whole cell extracts (10 - 20 μ g) of *P. gingivalis* isolates was separated on 8% SDS - PAGE, the gels were washed and incubated in enzyme reaction buffer for 18 h at 37 and stained with Coomassie blue and destained. Reference strains of *P. gingivalis* were included: ATCC(ATCC 33277),

				. KpnI	brillin				
	I			PstI	<i>P. gingivalis</i> 2561	fimbrillin(anti 2561			
	3가	(e, f	g)		fimbrillin Ab)	immunoblot		ATCC	
subgroup	8가	ribotype			33277	2561	43 kDa	fim -	
	. Ribotype I/e가	가			fimbrillin	49 kDa		A7A1	
(no 5, no7)					fimbrillin	2561 fimbrillin Ab		W50	anti
ribotype				2가					
2.								fimbrillin	
	ribotype						41 kDa(lane 5, 6, 8, 9, 10), 43		
SDS - PAGE							kDa(lane 2, 7) 49 kD(lane 1, 3, 4)		
40 kDa	60 kDa			2	F1	B2	fimbrillin	49 kDa	43
				가	kDa	fimbrillin	가		
	ribotype	가			4.				
ribotype	가								
	G2	B2			Gelatin		zymography		P.
					<i>gingivalis</i>				
							120 kDa	200 kDa	
3. Fimbrillin					가			200 kDa	
					가				
<i>P. gingivalis</i>	ribotype			fim -					

IV.

P. gingivalis, *P. gingivalis*
*P. gingivalis*가 16S
 9,45 - 47),
 rRNA
P. gingivalis
 , multilocus enzyme elec-
 trophoresis, ribotyping
 가
P. gingivalis
 가 , 가 *P. gingivalis* P.
 가 , *P. gingivalis*
 가
P. gingivalis
 . Ali
 1997 Ali , ,
P. gingivalis 198
 , ribotyping 32 ribotype
⁵³⁾. 1993 Loos 11
P. gingivalis 88 multilocus
 electrophoresis 68
 24).
P. gingivalis 39).
 6 10
 36 8가 ribo-
 type
 25).
P. gingivalis 가 ribotype
 . 1992 Socransky
 , *P. gingivalis* 6 *P. gingivalis*
 가 .
 25 ribotyping
P. gingivalis ribotype
 ribotype
 가 ribotype
 54). Ali 52 3
 2 ribotype ⁵³⁾ 1993
 Van Steenberg 8
 가 . 1 ⁴¹⁾, 1993 Saarela
P. gingivalis 9 1 2 가

55).
 ribotype cytokine
 가 가 P. gingi -
 1 Clonal turnover valis 381 fimbriae가
 가 Clone Clone fimbriae fimbrillin 가
 43 kDa
 KpnI I 1991 P. gingivalis
 PstI 3가 (e, f) fimbrillin
 g) 가 ribotype 40.5 49 kDa
 가
 subgroup Ali 23). 41 kDa, 43 kDa,
 10 4 ribotype 49 kDa fimbrillin
 /e 40%가 ribotype fimbrillin
 Ali 53)
 52 11.5%가 ribotype brillin
 가 Gelatin zymography
 11.5% ribotype 7/a가 가 P. gingivalis
 ribotype I/e P. gingivalis pro -
 Ali
 32 ribotype 13,63) protease
 8 ribotype 가
 cytokine , ,
 ATCC33277 W50 proteinase matrix metallo -
 ribotype band , valis P. gingi -
 KpnI ribotype 64). P. gingivalis
 Ali 32 ribotype
 P. gingivalis ribotype
 ribotype fimbrillin
 P. gingivalis 2561 10
 fimbrillin immunoblot P. gingivalis
 . P. gingivalis가
 56 - 58) fimbriae P. ribotype /e 가 40%
 gingivalis 2 ribotype
 가 59 - 62). fim -
 briae 가 P. gingivalis ribotyping

VI.

ribotype
ribotyping

V.

P. gingivalis ribotype
P. gingivalis ,
ribotype fimbriillin

1. Kpn I Pst I
P. gingivalis ribotyping 6
7 ribotype
Kpn ribotype
Pst
8 ribotype

2. Ribotype /e 가 가

3. ribotype

4. P. gingivalis
fimbriillin 41KDa, 43KDa, 49KDa

5. P. gingivalis
120KDa
10
P. gingivalis
16S rRNA
ribotyping 가
ribotype /e 40%
2 ribotype

1. Moore W.E.C. and Moore L.V.H. : The bacteria of periodontal diseases, Periodontol. 2000, 5: 66 - 77, 1994.
2. Holt S.C. and Bramanti T. : Factors in virulence expression and their role in periodontal disease pathogenesis, Crit. Rev. Oral. Biol. Med. 2: 77 - 81, 1991.
3. Slots J. and Genco R.J. : Black - pigmented Bacteroides species, Capnocytophaga species, and Actinobacillus actinomycetemcomitans in human periodontal disease virulence factors in colonization, survival, and tissue destruction, J. Dent. Res., 63:412 - 421, 1984.
4. Dahl n G. : Black - pigmented gram - negative anaerobes in periodontitis. FEMS Immunol. Med. Microbiol., 6:181 - 192, 1993.
5. Haffajee A.D. and Socransky S.S. : Microbial etiological agents of destructive periodontal diseases, In: Socransky S.S. and Haffajee A.D. ed. Microbiology and immunology of periodontal disease. Periodontol. 2000., 5:74 - 111, 1994.
6. Loesche W.J., Syed S.A., Schmidt E. and Morrison E.C. : Bacterial profiles of subgingival plaques in periodontitis, J. periodontol., 56: 447 - 56, 1985.
7. Slots J. : Bacterial specificity in adult periodontitis. A summary of recent work, J. Clin. Periodontol., 13:570 - 7, 1986.
8. Sundqvist G. : Pathology and virulence of black - pigmented gram - negative anaerobes in periodontitis. FEMS Immunol. Med. Microbiol., 6:125 - 138,

- 1993.
9. Tanner A.C.R., Socransky S.S. and Goodson J.M. : Microbiota of periodontal pockets losing crestal alveolar bone, *J. Periodont. Res.*, 19:279 - 291, 1984.
 10. Holt S.C., Kesavalu L. walker S. and Genco C.A.: Virulence factors of porphyromonas gingivalis, *Periodontol.*2000, 20:168 - 238, 1999.
 11. Mayrand D. and Holt S.C. : Biology of asaccharolytic black - pigmented Bacteriodes species, *Microbiol. Rev.*, 52: 134 - 152, 1988.
 12. Shah H.N. : Biology of the species Porphyromonas gingivalis. Ann Arbor. MI: CRC Press., 1993.
 13. Kuramitsu H. K. : Protease of Porphyromonas gingivalis : what don't they do?, *Oral Microbiol. Immunol.* : 13: 263 - 270, 1998.
 14. Lewis J.p. and Macrina F.L. : IS/95, an insertion sequence - like element associated with protease genes in Porphyromonas gingivalis, *Infect. Immun.*, 3035 - 3042, 1998.
 15. Beachey, E. H. : Bacterial adherence : adhesin - receptor interactions mediating the attachment of bacteria to mucosal surfaces, *J. Infect. Dis.* 143:325 - 345, 1981.
 16. Gibbons R. J. : Adherent interactions which may affect microbial ecology in the mouth. *J. Dent. Res.*, 63:378 - 385, 1984.
 17. Gibbons, R. J., and van Houte : Bacterial adherence and the formation of dental plaques, P. 61 - 104. In E. H. Beachey (ed.), *Bacterial adherence: receptors and recognition*, series B, vol. 6. Chapman & Hall, London, 1980.
 18. Hamada S., Fujiwara T., Morishima S., et al. : Molecular and immunological characterization of the fimbriae of porphyromonas gingivalis, *Microbiol. Immunol.*, 38:921 - 930, 1994.
 19. Jones G. W. : The attachment of bacteria to the surfaces of animal cells, P. 139 - 176. In J. L. Ressig. (ed.), *Microbial interactions: receptors and recognition*, series B, vol. 3. Chapman & Hall, Ltd., London, 1977.
 20. Ofek I. and Beachey E. H. : General concepts and principles of bacterial adherence in animal and man, P. 1 - 29. In E. H. Beachey (ed.), *Bacterial adherence: receptors and recognition*, series B, vol. 6. Chapman & Hall, Ltd., London, 1980.
 21. Gaastra W., and de Graaf. F. K. : Host - specific fimbrial adhesins of non - invasive enterotoxigenic Escherichia coli strains. *Microbiol. Rev.* 46:129 - 161. 1982.
 22. Germanier R. :(ed.) *Bacterial vaccines*. Academic Press Inc., New York, 1984.
 23. Lee J. Y., Sojar H. T., Bedi G. S. and Genco R. J. : Porphyromonas(Bacteroides) gingivalis Fimbrillin : size, amino terminal sequence, and antigenic Heterogeneity, *Infect. Immun. Jan* : 383 - 389, 1991.
 24. Ali R.W., Bakken V., Nilsen R. and Skaug N. : Comparative detection frequency of six putative periodontal pathogens in Sudanese and Norwegian adult periodontitis patients, *J. Periodontol.*, 65:1046 - 1052, 1994.

25. Ali R.W., Velcescu C., Jivanescu M., Lofthus B. and Skaung N. : Prevalence of six putative periodontal pathogens in subgingival plaque samples in Romanian adult periodontitis patients, *J. Clin. Periodontol.*, 23:133 - 139, 1996.
26. Dahl n G., Manji F., Baelum V. and Fejerskov O. : Black - pigmented *Bacteroides* species and *Actinobacillus actinomycetemcomitans* in subgingival plaque of adult Kenyans, *J. Clin. Periodontol.*, 26:305 - 310, 1989.
27. Moore W.E.C., Holdeman L.V., Cato E.P., Smibert R.M., Burmeister J.A. and Ranney R.R. : Bacteriology of moderate(chronic) periodontitis in mature adult humans, *Infect. Immun.*, 42: 510 - 515. 1983.
28. Slots J., Rams T.E., Feik D., Taveras H.D. and Gillespie M. : Subgingival microflora of advanced periodontitis in the Dominican Republic, *J. Periodontol.*, 62:543 - 547, 1991.
29. Tanner A.C.R., Haffer C., Bratthall G.T., Visconti R.A. and Socransky S.S. : A study of the bacteria associated with advancing periodontitis in man, *J. Clin. Periodontol.*, 6:278 - 307, 1979.
30. Lalibert M. and Mayrand D. : Characterization of black - pigmented *Bacteroides* strains isolated from animals, *J. Appl. Microbiol.*, 55:247 - 252, 1983.
31. Nagata A., Man - yoshi T., Sato M. and Nakamura R. : Serological of *Porphyromonas*(*Bacteroides*) *gingivalis* and correlation with enzyme activity, *J. Periodont. Res.*, 26: 184 - 190, 1991.
32. Notten F.J.W., Nieman F.H.M. and Mikx F.H.M. : Antibiotypes of *Bacteroides gingivalis* assessed by antimicrobial disks and multivariate analysis, *J. Clin. Microbiol.*, 22: 1020 - 1024, 1985.
33. Kinder S. A., Korman K.S. and Holt S.C. : Characterization of selected gram - negative oral microorganisms by SDS - PAGE, *Oral. Microbiol. Immunol.*, 4:52 - 57, 1989.
34. Laemmli U. K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature(London)* 227:680 - 685. 1970 .
35. Madianos P.N., Papapunou P.N., Socransky S.S., Dahl n G. and Sandros J. : Host - related genotypic heterogeneity of *Porphyromonas gingivalis* strains in the beagle dog, *Oral Microbiol. Immunol.*, 9: 241 - 247, 1994.
36. Teanpaisan R., Douglas C.W.I., Eley A.R. and Walsh T.F. : Clonality of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens* isolated from periodontally diseased and healthy sites, *J. Periodont. Res.*, 31:423 - 432, 1996.
37. Zhang Y.J., Yasui S., Yoshimura F. and Ishikawa I. : Multiple restriction fragment length polymorphism genotypes of *Porphyromonas gingivalis* in single periodontal pockets, *Oral Microbiol. Immunol.*, 10:12 - 128, 1995.
38. Loos B. G. and Dyer D. W. : Restriction fragment length polymorphism analysis of the fimbrillin locus, *fimA*, of *Porphyromonas gingivalis*, *J. Dent. Res.*, 71: 1173 - 1181, 1992.
39. Loos B. G., Dyer D. W., Whittam T.

- S. and Selander R. K. : Genetic structure of populations of *Porphyromonas gingivalis* associated with periodontitis and other oral infections, *Infect. Immun.*, 61: 204 - 212, 1993.
40. Loos B. G., Mayrand D., Genco R. J. and Dickinson D. P. : Genetic heterogeneity of *Porphyromonas*(*Bacteroides*) *gingivalis* by genomic DNA fingerprinting [published erratum appears in *J Dent Res* 1990 Sep : 69(9) : following 1323]. *J. Dent. Res.*, 69: 1488 - 1493, 1990.
41. Van Steenberg T.J.M., Petit M.D.A., Scholte L.H.M., van der Velden U. and de Graaff J. : Transmission of *Porphyromonas gingivalis* between spouses, *J. Clin. Periodontol.*, 20:340 - 345, 1993.
42. Sims T.J., Ali R.W., Brockman E.S., Skaug N. and Page R.C. : Antigenic variation in *Porphyromonas gingivalis* ribotypes recognized by serum immunoglobulin G of adult periodontitis patients, *Oral Microbiol. Immunol.*,

- 14:73 - 85, 1999.
43. Syed S.A. and Loesche W.J. : Survival of human dental plaque flora in various transport media, *Appl.Microbiol.*, 24:638 - 644, 1972.
 44. , , , , : 16S rRNA , 28: 691 - 701,1998.
 45. Rodenburg J.P., van Winkelhoff A.J., Winkel E.G., Goene R.J., Abbas F. and Graaff J. : Occurrence of *Bacteroides gingivalis*, *Bacteroides intermedius*, and *Actinobacillus actinomycetemcomitans* in severe periodontitis in relation to age and treatment history, *J. Clin Periodontol.*, 17:392 - 399, 1990.
 46. Van Steenberg T.J.M., van Winkelhoff A.J. and de Graff J.: Black - pigmented oral anaerobic rods: classification and role in periodontal disease, In Hamada S, Holt S.R.(ed), *Periodontal diseases: pathogens and host immune responses*, Quintessence Publishing Company,Ltd., Tokyo, p41 - 52, 1991.
 47. Van Winkelhoff A.J., Van der Velden U., Clement M. and de Graaff J.: Intra - oral distribution of black - pigmented *Bacteroides* species in periodontal patients, *Oral Microbiol. Immunol.*, 3:83 - 85, 1988.
 48. Grenier D. and Mayrand D. : Selected characteristics of pathogenic and non - pathogenic strains of *Bacteroides gingivalis*, *J. Clin. Microbiol.*, 25:738 - 740, 1987.
 49. Mckee A.S., Mcdermid A.S., Wait R., Baskerville A. and Marsh P.D.: Isolation of colonial variance of *Bacteroides gingivalis* W50 with a reduced virulence, *J. Med. Microbiol.*, 27: 59 - 64, 1989.
 50. Neiders M.E., Chen P.B., Suido H. Reynolos H.S. and Zambon J.J. : Heterogeneity of virulence among strains of *Bacteroides gingivalis*, *J. Periodont. Res.*, 24: 192 - 198, 1989.
 51. Sundqvist G., Figdor D., Hanstrom L., Sorlin S. and Sandstrom G. : Phagocytosis and virulence of different strains of *Porphyromonas gingivalis*, *Scand. J. Dent. Res.*, 99:117 - 129, 1991.
 52. Van Steenberg T.J.M., Delemarre F. G. A., Namavar F. and de Graaff J. : Differences in virulence within the species *Bacteroides gingivalis*. *Antonie Van Leeuwenhoek*, 53:233 - 244, 1987.
 53. Ali R.W., Martin L., Haffajee A.D. and Socransky S. S. : Detection of identical ribotypes of *porphyromonas gingivalis* in patients residing in the United States, Sudan, Romania and Norway, *Oral Microbiol. Immunol.*, 12:106 - 111, 1997.
 54. Socransky S. S. and Martin L. : Ribotyping of *Porphyromonas Gingivalis* and *Campylobactor rectus*. *J. Dent. Res.*, 71:246, 1127, 1992.
 55. Saarela M., Von Troil - Linden B., Torkko H. et al. : Transmission of oral bacterial species between spouses, *Oral. Microbiol. Immunol.*, 8:349 - 354, 1993.
 56. Hamada S. Amano A., Kimura S., Nakagawa I. Kawabata S. and Morisaki I. : The importance of fimbriae in the virulence and etiology of some oral bacteria, *Oral Microbiol. Immunol.*, 13:129 - 138, 1998.

57. Malek R., Fisher J.G., Caleca A., Stinson M., Vanos C.J. Lee J.Y., Cho M. I., Genco R.J., Evans R.T. and Dyer D.W.: Inactivation of the porphyromonas gingivalis fimA gene blocks periodontal damage in gnotobiotic rats. *J. Bacteriol.*, 176: 1052 - 1059, 1994.
58. Okuda K. : Attachment mechanisms and colonization, IN shah H.N. Mayrand D. and Genco R.J.(ed), *Biology of the species Porphyromonas gingivalis*. C.R.C. Press, Inc., Boca Ration, Fla., P. 139 - 158, 1993.
59. Okuda K. and Takazoe I. : Haemagglutinating activity of *Bacteroides melaninogenicus*. *Arch. Oral Biol.*, 19: 415 - 416, 1974.
60. Okuda K., Slots J. and Genco R. J. : *Bacteroides gingivalis*, *Bacteroides asaccharolyticus*, and *Bacteroides melaninogenicus* subspecies: cell surface morphology and adherence to erythrocytes and human buccal epithelial cells, *Curr. Microbiol.*, 6: 7 - 12, 1981.
61. Slots J., and Gibbons R.J. : Attachment of *Bacteroides melaninogenicus* subsp. *asaccharolyticus* to oral surfaces and its possible role in colonization of the mouth and periodontal pockets. *Infect. Immun.*, 19;254 - 264, 1978.
62. Woo, D. D. L., Holt S. C. and Leadbetter E. R. : Ultrasturcture of *Bacteroides* species: *Bacteroides asaccharolyticus*, *Bacteroides fragilis*, *Bacteroides melaninogenicus* subspecies *intermedius*. *J. Ingect. Dis.*, 139:534 - 546, 1979.
63. Potempa J., Pike R. and Travis J. : Host and *Porphyromonas gingivalis* proteases in periodontitis : a biochemical model of infection and tissue destruction, *Perspect Drug. Discovery Design*, 2:445 - 458, 1995.
64. Slots J. : Enzymatic characterization of some oral and nonoral gram-negative bacteria with the A.P.I. Z.Y.M. system, *J. Clin Microgiol.* 14: 288 - 294, 1981.

- Abstract -

Ribotyping of Porphyromonas Gingivalis Isolated from Rapidly Progressive Peri- odontitis Patients

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This study examined ribotypes of 36 *P. gingivalis* strains isolated from 10 rapidly progressive periodontitis patients in Korean and revealed the presence of genetic heterogeneity among the patients. Ribotyping was performed by using a oligonucleotide probes based on 16S rRNA after whole genomic DNA had been digested with the restriction endonuclease enzyme Kpn I and Pst I. In addition, the antigenic heterogeneity of fimbrillin and protease activity was analysed to observe the virulency of *P. gingivalis*. The results were as follows.

1. Using KpnI, 6 ribotypes were detected, whereas 7 ribotypes were identified by using PstI. When combined two enzymes, a total of 8 ribotypes was subgrouped.

2. Ribotype /e was the most common and detected in 4 among 10 patients.

3. The fimbrillin expressed from *P. gingivalis* isolates had the molecular size of 41kDa, 43kDa, 49kDa. It was observed that the size of fimbrillin with the same ribotypes could be identical.

4. All the *P. gingivalis* strains showed strong proteolytic activity and had the molecular size more than 120kDa.

In summary, total 8 ribotypes were observed for isolates from rapidly progressive periodontitis patients. Forty percent of the patients harbored isolates exhibiting the same ribotype /e, and it was observed that more than one ribotype can coexist in an individual patient.