

Porphyromonas gingivalis ribotyping

1 . 2 . 1 . 1 . 1 . 1
 , 1
 2

I.

15 20 500
 P. gingivalis 15-20 fimbriae 가
 . Fimbriae 가 21,22)
 fimbrillin , fimbriae
 가 23)
 가 ,
 P. gingivalis 24-29)
 가
 P. gingivalis
 가
 P. gingivalis
 2,3),
 P. gingivalis
 P. gingivalis
 4-9), P. valis
 10-12)
 13,14),
 (phenotypic method)
 (genotypic method)
 Biotyping³⁰⁾, serotyping³¹⁾, antibiotyping³²⁾
 SDS - PAGE protein profile^{33,34)}

가 rRNA) 가
16S rRNA ,

P. gingivalis
, multilocus enzyme elec-
trophoresis ribotyping P. gingivalis , ,
P. gingivalis P. gingivalis
35 - 37), P. gingivalis vaccine
가 P. gingivalis 42) 가 P.
P. gingivalis gingivalis 가 P.
38 - 41), Loos
DNA P. gingivalis
(restriction fragment length polymorphism:
RFLP) ribotype 가
P. gingivalis P. gingivalis

33
P. gingivalis 29가 DNA 16S rRNA
RFLP 40). Van ribotyping
Steenbergen P. gingivalis가 ribotype fimbrillin
가 ,
가 P. gingivalis
DNA 가 II.
DNA
41). Loos Dyer가 P. gin - 1 .
givalis 100 multilocus Y
enzyme electrophoresis 10
78가
39). 5 22
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

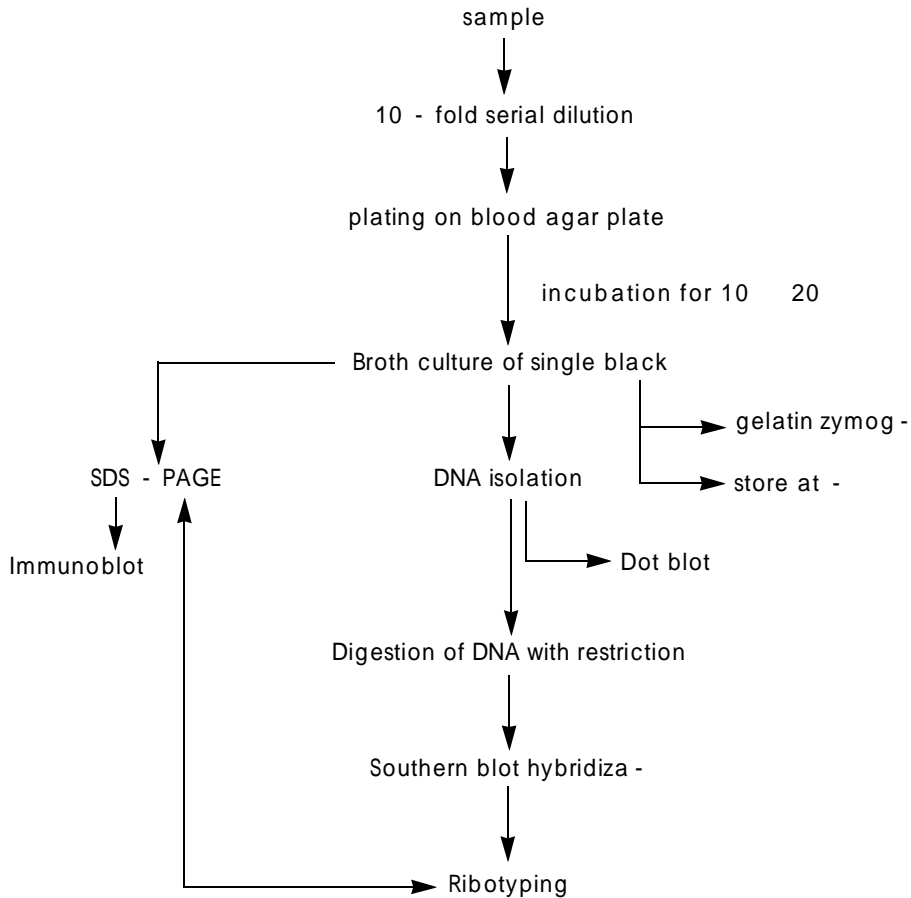
* CSPD

(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 west - ern 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

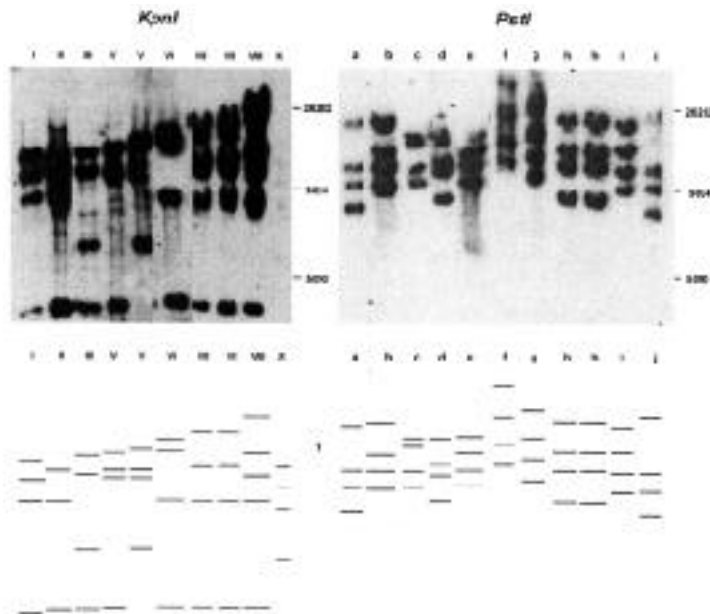


Figure 1. Ribotype patterns of representative *P. gingivalis* strains isolated from RPP patients and 4 reference strains. DNA of the isolates was digested with restriction enzymes Kpn I and Pst I, respectively and probed with a 16S rRNA - specific oligonucleotide probe for *P. gingivalis*. Reference 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:

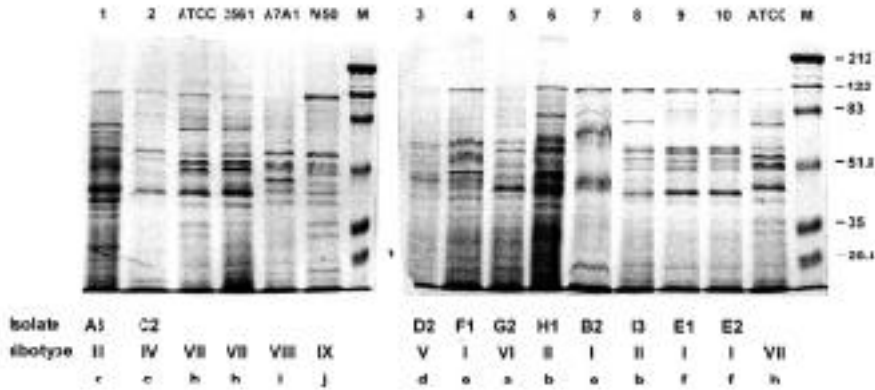


Figure 2. Coomassie blue - stained SDS - PAGE separation patterns of whole cell sonicates(10 - 20µg) of representative *P. gingivalis* isolates using 10% polycarylamide gel. Reference strains of *P. gingivalis* were included: ATCC(ATCC 33277), 2561(ATCC 33277), A7A1 - 28, W50(ATCC

dH₂O(1:1:8)

oligonucleotide

KpnI PstI DNA

III.

1. *P. gingivalis* ribotype

6, PstI (Figure 1), 7

36 *P. gingivalis* 10 . *P.* ATCC ribotype

gingivalis . KpnI 10 5

16S rRNA I 2 II ,

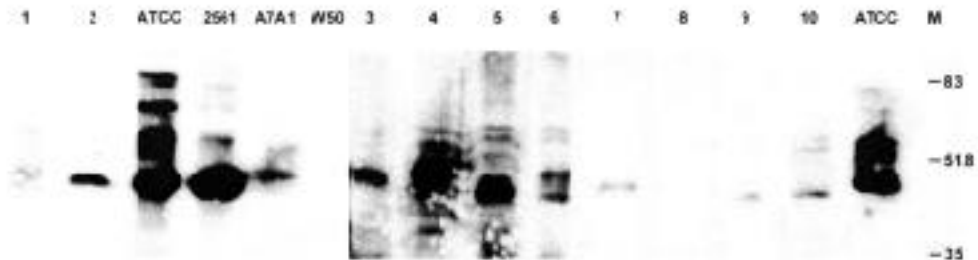


Figure 3. Immunoblot of whole cell sonicates of *P. gingivalis* isolates with polyclonal antibodies directed against fimbrillin of *P. gingivalis* 2561. After whole cell extracts(10 - 20µg) of *P. gingivalis* isolates was separated on 10% SDS - PAGE, proteins were transferred to nitrocellulose membranes 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 -

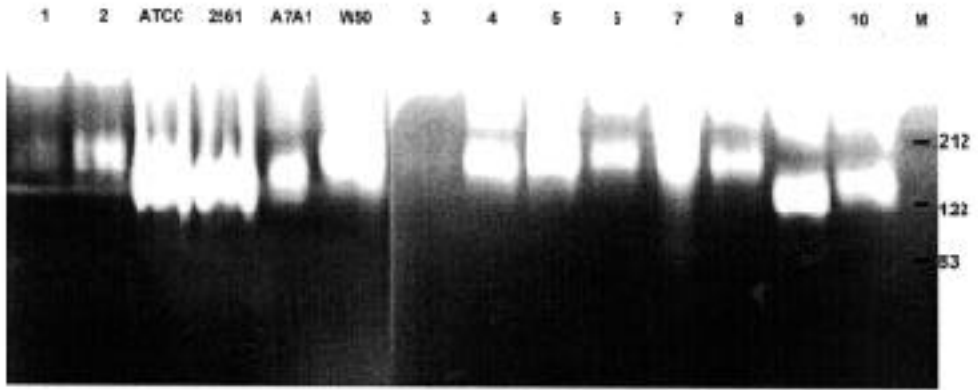


Figure 4. Zymograms of whole cell sonicates of *P. gingivalis* isolates. After whole cell extracts(10 - 20 μ g) of *P. gingivalis* isolates was separated on 8% SDS - PAGE, the gels were washed and incubated in enzyme reaction buffer for 18 h at 37 and stained with Coomassie blue and destained. Reference 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	
				2561 fimbrillin Ab			W50	anti
(no 5, no7)			2가					
ribotype								
2.						fimbrillin		
						41 kDa(lane 5, 6, 8, 9, 10), 43		
						kDa(lane 2, 7) 49 kD(lane 1, 3, 4)		
	ribotype					ribotype(ribotype I/e)	가	
SDS - PAGE			2	F1	B2	fimbrillin	49 kDa	43
40 kDa	60 kDa		가	kDa	fimbrillin	가		
	ribotype	가		4.				
가								
ribotype	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. gingivalis가				16S
	9,45 - 47),		rRNA	
			P. gingivalis	
가			, multilocus enzyme elec - trophoresis, ribotyping	
	P. gingivalis			
가	,	가	P. gingivalis	P.
	가		gingivalis	
	P. gingivalis		1997 Ali	
. Ali			,	
	P. gingivalis,		P. gingivalis 198	
Prevotella intermedia, Actinobacillus actin - omycetemcomitans, Fusobacterium nuclea - tum Capnocytophaga	,		, ribotyping 32	ribotype
DNA			⁵³⁾ . 1993 Loos	11
			P. gingivalis 88	multilocus
			electrophoresis	68
	24).		³⁹⁾ .	
	P. gingivalis		10	
6			36	8가 ribo - type
25).			가	ribotype
P. gingivalis			1992 Socransky	
, P. gingivalis			6	P. gingivalis
가			25	ribotyping
P. gingivalis				ribotype
				ribotype
	P. gingivalis		가	ribotype
26,48 - 52).			⁵⁴⁾ . Ali	52 3
			2	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
 KpnI I 43 kDa
 PstI 3가 (e, f) 1991 P. gingivalis
 g) 가 ribotype 40.5 49 kDa
 subgroup Ali 23). 41 kDa, 43 kDa,
 10 4 ribotype 49 kDa fimbrillin
 /e 40%가 ribotype 53) ribotype fim -
 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

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- 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.