

Prevotella intermedia

Prevotella nigrescens

.

	I.	
intermedia	Prevotella 1,2) ANUG ³⁾ , 가	Prevotella intermedia 가 homology group Prevotella intermedia Prevotella nigrescens ¹⁰⁾ .
4)	가	, , Prevotella intermedia Prevotella nigrescens DNA homology (homology group 4197 Prevotella intermedia , homology group 8944 Prevotella nigrescens .) ⁸⁾ ,
5)	가	serology (serogroup I Prevotella inter - media , serogroup II III Prevotella nigrescens .) ⁹⁾ , multilocus enzyme electrophoresis ¹⁰⁾ , sodium dodecyl sulfate - polyacrylamide gel electrophore - sis (SDS - PAGE) protein profile ¹¹⁾ ,
가	6,7), 가	oligonucleotide probe ¹²⁾ genomic DNA hybridization ^{13,14)}
Prevotella intermedia	intraspecies het - erogeneity Johnson Holdeman	, , Prevotella intermedia 가 가 DNA homology group 가
8),	Gm r Guggenheim	가 , Polymerase Chain
monoclonal antibody	Prevotella	Reaction (PCR)
intermedia 3	serogroup (I, II, III)	, ^{15,16)}
multilocus enzyme electrophoresis	9), Shah Gharbia	Prevotella intermedia type strain

* 1997

ATCC 25611 *Prevotella nigrescens*
type strain ATCC 33563

Prevotella intermedia *Prevotella nigrescens*가

. Gharbia

Prevotella intermedia

Prevotella intermedia *Prevotella nigrescens*

Prevotella intermedia

Prevotella nigrescens

Prevotella intermedia

Prevotella intermedia (70.7%),
26.7% *Prevotella intermedia*
.11)

, Teanpaisan

Gharbia

Prevotella intermedia 가 18%,
Prevotella nigrescens 가 31%

Prevotella nigrescens 가
Prevotella intermedia

Prevotella intermedia 가

.17)

Prevotella intermedia

Prevotella nigrescens

DNA sequence
Reaction(PCR)

16S ribosomal
Polymerase Chain

II.

1. Sample

Table 1. Biochemical characteristics of BPB

	glucose	sucrose	lactose	cellbiose	esculin	indole
<i>P. gingivalis</i>	-	-	-	-	-	+
<i>P. intermedia</i>	+	V	-	-	-	+
<i>P. melaninogenicus</i>	+	+	+	-	-	-
<i>P. loeshii</i>	+	+	+	+	+	-
<i>P. levii</i>	+	-	+	-	-	-
<i>P. oralis</i>	+	+	+	+	+	-

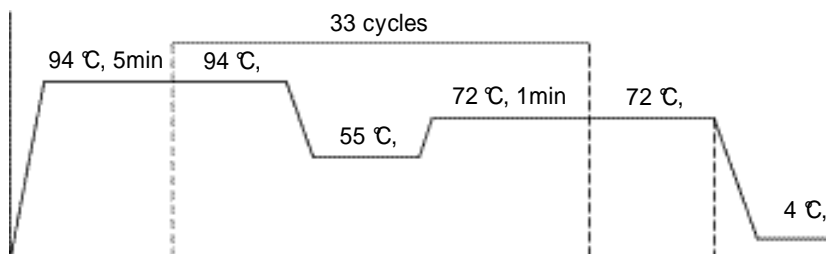


Figure 1. PCR procedure

6 intermedia Prevotella nigrescens
 . Prevotella intermedia
 100 BHI
 50 가 pellet . pellet 5
 5mm guanidine
 pocket depth 가 phenol chloroform DNA
 . sample , EtOH DNA DNA
 , DNA 70%
 #35 paper point , EtOH TE(pH 8.0)
 DNA 4°C
 가 DNA UV - spectrophotometer
 , 2ml VMGA III primer
 10% H₂, 10% CO₂, 80% N₂ design¹⁸⁾ primer
 2. DNA Accu™ Power
 Premix kit PCR machine
 1 . PCR
 1000 10 DNA ethidium bromide가 가 1.0%
 hemin menadione 가 agarose gel 80V 40
 . 37°C 10 UV transilluminator
 colony black - pigmented bacteria band . size marker
 BHI Gram 100bp DNA ladder(Life technologies Co.
 biochemical test(Table 1) U.S.A.) polaroid camera
 Prevotella intermedia (Seoulin scientific Co. LTD., Korea)
 III.
 3. 100
 sample 39 sample BPB가
 Prevotella intermedia 24 가 Prevotella intermedia
 Prevotella . Prevotella intermedia

Table 2. The frequency of P.intermedia and P.nigrescens in infected periodontal tissue and normal periodontal tissue

	Total samples	BPB detecting samples	P.intermedia detecting samples	P.nigrescens detecting samples
Infeted periodontal tissue	100	39	21	2
Normal periodontal tissue	50	9	1	1

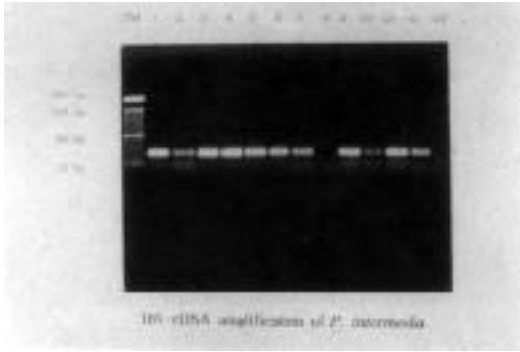


Figure 2. 16S - rDNA amplification of *Prevotella intermedia*

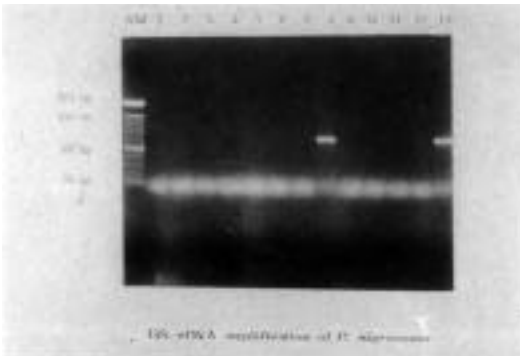


Figure 3. 16S - rDNA amplification of *Prevotella nigrescens*

agarose gel
 , 24 *Prevotella intermedia* 2
 가 *Prevotella nigrescens* primer
 830bp , 21
 가 *Prevotella intermedia* primer
 240bp .
 1 primer
 .
 50
 sample 9 sample BPB가
 2 가 *Prevotella intermedia*
 ,

Prevotella intermedia
Prevotella nigrescens

Figure 2 3 PCR
Prevotella intermedia *Prevotella nigrescens*
 , Table 2

IV.

BPB *Actinobacillus actinomycetem - comitans* 가

Bacteroides 가

cytophaga - flavobacter - bacteroides(CFB) phylum subgroup 30

가 , BPB
 genus *Bacteroides*

Shah Collins genus *Bacteroides* 3가 genera

; 1)saccharolytic, non - pigmented species *Bacteroides* (e.g. *B. fragilis*), 2)asaccharolytic, black - pigmenting species *Porphyromonas* (e.g. *P. gingivalis*), 3)saccharolytic, black - pigmenting species *Prevotella* (e.g. *P. intermedia*)¹⁹⁾. *Porphyromonas* species 6 *Prevotella* species

protoporphyrin protoheme pigment .²⁰⁾
Prevotella intermedia gram - negative,

obligately anaerobic rod

Actinobacillus actinomycetemcomitans
Porphyromonas gingivalis
Prevotella intermedia

Fusobacterium nucleatum

Prevotella intermedia

²¹⁾

Prevotella nigrescens 1992 Shah
Gharbia Prevotella intermedia

Prevotella nigrescens gram - negative, non - sporing,
obligately anaerobic, rod

Broth culture 0.3 0.7µm 1
2µm . Blood agar

3 surface colony 0.5
2mm low convex

colony . Pigmentation

Glucose가 broth
acetic, isobutyric, isovaleric,
succinic acid . Dextran glucose,
maltose, sucrose strain

fructose glycogen, insulin
가 .¹⁰⁾

Prevotella intermedia
Porphyromonas gingivalis

(surface property) 가

, lipopolysaccharide, capsule,
surface appendage

Prevotella nigrescens

Prevotella intermedia
Prevotella nigrescens

Prevotella intermedia surface
appendage가 가 type

가 ²²⁾, negative staining

Prevotella intermedia

fibriae - like projection

Prevotella nigrescens

EM Prevotella nigrescens
capsular layer가 Prevotella intermedia

¹¹⁾. Prevotella inter -
media Prevotella nigrescens

가

Prevotella intermedia Prevotella
nigrescens transmission

, 가 trans -
mission

^{23,24)}.

Prevotella intermedia Prevotella
nigrescens site specificity

1994 Gharbia

Prevotella intermedia

Prevotella nigrescens

Prevotella nigrescens가

Prevotella intermedia가

(70.7%) ¹¹⁾, 1997

Prevotella intermedia

Prevotella nigrescens

Prevotella nigrescens

(73.2%)

²⁵⁾. 1995 Teanpaisan

BPB Prevotella

intermedia Prevotella nigrescens

Prevotella nigrescens

sample Prevotella intermedia
Prevotella nigrescens가 1

Prevotella nigrescens

Prevotella intermedia

가

17),

Prevotella

intermedia Prevotella nigrescens site
specificity 가

가

(11,17,26,27 - 29)

가

,가

transmission

100 sample 39 BPB가
24 가

false positive effect가 가
, 가

Prevotella intermedia
16s

DNA

가

24 sample 21 가 Prevotella
intermedia 2 가 Prevotella nigrescens
, 1 sample

BPB

Gharbia

11).

Prevotella nigrescens

Prevotella intermedia가

Actinobacillus actinomycetemcomitans

JP - 2 like gene 가

30)

가

Prevotella inter -

Teanpaisan

media Prevotella nigrescens

가

Prevotella
nigrescens가 Prevotella intermedia
(37.7% : 15.5%),

Prevotella intermedia Prevotella
nigrescens multilocus enzyme elec -
trophoresis monoclonal antibody, protein
profile

Porphyromonas gingivalis

Prevotella intermedia Prevotella
nigrescens

가

가

17).

1991 Gurtler
DNA

16s

50

Prevotella inter -

media Prevotella nigrescens

paper point

sample

15,16)

가

test

Prevotella intermedia

biochemical

intermedia Prevotella nigrescens

Prevotella

Prevotella intermedia
nigrescens

Prevotella

Prevotella intermedia

Prevotella nigrescens

1.

, 100

가

100

sample 39

Prevotella intermedia
nigrescens가 site specificity

Prevotella
가

sample (39%)

BPB가

2.

, BPB가

39

가 가

가 ,

sample 24
intermedia

sample

Prevotella

3.

, Prevotella inter -

media 24

sample 21

Prevotella intermedia(87.5%), 2

Prevotella nigrescens (8.33%)

가

Prevotella

4.

, 50

nigrescens가 Prevotella intermedia

50

sample 9

sample BPB가

1 sample Prevotella intermedia, 1

sample Prevotella nigrescens

가

가

VI.

V.

1. Slots J, Bargd L, Wikstrom M, Dahlen G. The occurrence of Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius in destructive periodontal disease in adults. J Clin Periodontol 1986;13:570 - 577

Prevotella intermedia

Prevotella nigrescens

, 6

가

2. Wennstrom JL, Dahlen G,

- Svensson J, Nyman S. Actinobacillus actinomycetemcomitans, Bacteroides gingivalis and Bacteroides intermedius: predictors of attachment loss? Oral Microbiol Immunol 1987;2:158 - 163
3. Loesche WJ, Syed SA, Laughon BE, Stoll J. The bacteriology of acute necrotizing ulcerative gingivitis. J Periodontol 1982;53:223 - 230
 4. Kornman KS, Loesche WJ. The subgingival microbial flora during pregnancy. J Periodont Res 1980;15:111 - 122
 5. Kononen E. Pigmented Prevotella species in the periodontally healthy oral cavity. FEMS Immunol Med Microbiol 1993;6:201 - 206
 6. Frisken KW, Higgins T, Palmer JM. The incidence of periodontopathic microorganism in young children. Oral Microbiol Immunol 1990;5:43 - 45
 7. Kononen E, Asikainen S, Saarela M, Karjalainen J, Jousimies - Somer H. The oral gram - negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth. Oral Microbiol Immunol 1994;9:136 - 141
 8. Johnson JL, Holdeman LV. Bacteroides intermedius comb. nov. and descriptions of Bacteroides corporis sp. nov. and Bacteroides levii sp. nov. Int J Syst Bacteriol 1983;33: 15 - 25
 9. Gmur R, Guggenheim B. Antigenic heterogeneity of Bacteroides intermedius as recognized by monoclonal antibodies. Infect Immun 1983;42:459 - 470
 10. Shah HN, Gharbia SE. Biochemical and chemical studies on strains designated Prevotella intermedia and proposal of a new pigmented species, Prevotella nigrescens sp. nov. Int J Syst Bacteriol 1992;42:542 - 546
 11. Gharbia SE, Haapasalo M, Shah HN et al. Characterization of Prevotella intermedia and Prevotella nigrescens isolates from periodontic and endodontic infections. J Periodontol 1994;65:56 - 61
 12. Dix K, Watanabe SM. et al. Species - specific oligodeoxynucleotide probes for the identification of periodontal bacteria. J Clin Microbiol 1990;28:319 - 323
 13. Fukushima H, Moroi H, Inoue J et al. Phenotypic characteristics and DNA relatedness in Prevotella intermedia and similar organism. Oral Microbiol Immunol 1992;7:60 - 64
 14. Moncla BJ, Strockbine L, Braham P, Karlinsey J, Roberts MC. The use of whole - cell DNA probes for the identification of Bacteroides intermedius isolates in a dot blot assay. J Dent Res 1988;67:1267 - 1270
 15. Gurtler V, Wilson VA, Mayall BV. Classification of medically important clostridia using restriction endonuclease

- site differences of PCR - amplified 16S rDNA. *J Gen Microbiol* 1991;137:2673 - 2679
16. Susan E. Milsom, Susan V. Sprague, D. Dymock. Rapid differentiation of *Prevotella intermedia* and *Prevotella nigrescens* by 16S rDNA PCR - RFLP. *J Med Microbiol* 1996;44: 41 - 43
 17. Teanpaisan R, Douglas CWI, Walsh TF. Characterization of black - pigmented anaerobes isolated from diseased and healthy periodontal sites. *J Periodont Res* 1995;30:245 - 251
 18. Bae KS, Baumgartner JC, Watkins BJ, Tian X. Association of black - pigmented bacteria with endodontic infections. Unpublished.
 19. Shah HN, Collins DM. *Prevotella*, a new genus to include to *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* 1990;40:205 - 208
 20. Van Winkelhoff AJ. Black - pigmented *Bacteroides* in human oral diseases. Amsterdam: Free University Press. 1986
 21. Raouf Wahab Ali, Nils Skaug, Rune Nilsen. Microbial associations of 4 putative periodontal pathogens in Sudanese adult periodontitis patients determined by DNA probe analysis. *J Periodontol* 1994;65:1053 - 1057
 22. Leung KP, Fukushima H, Sagawa H, Walker CB, Clark WB. Surface appendages, hemagglutination, and adherence to human epithelial cells of *Bacteroidis intermedius*. *Oral Microbiol Immunol* 1989;4:204 - 210
 23. Matto J, Saarela M, Jousimies - Somer H, Torkko H, Asikainen S. Distribution and genetic analysis of oral *Prevotella intermedia* and *Prevotella nigrescens*. *Oral Microbiol Immunol* 1996;11:96 - 102
 24. Van Steenberghe TJM, Basch - Tijhof CJ, Petit MDA, Van de Velden. Intra - familial transmission and distribution of *Prevotella intermedia* and *Prevotella nigrescens*. *J Periodont Res* 1997;32:345 - 350
 25. Bae KS, Baumgartner JC. Occurrence of *Prevotella nigrescens* and *Prevotella intermedia* in infections of endodontic origin. *J Endod.* 1997;23:620 - 623
 26. Gmur R, Guggenheim B. Interdental supragingival plaque - a natural habitat of *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Campylobacter rectus*, and *Prevotella nigrescens*. *J Dent Res* 1994;73:1421 - 1428
 27. Dahlen G, Wikstrom M, Renvert S, Gmur R, Guggenheim B. Biochemical and serological characterization of *Bacteroides intermedius* strains isolated from the deep periodontal pocket. *J Clin Microbiol* 1990;28:2269 - 2274
 28. Moore LVH, Moore WEC, Cato et al. Bacteriology of human gingivitis. *J Dent Res* 1987;66:989 - 995
 29. Moore WEC, Moore LH, Ranney RR, Smibert RM, Burmeister JA, Schenkein HA. The microflora of periodontal sites showing active destructive progression. *J Clin Periodontol* 1991;18:729 - 739

30. Haubek D, Dirienzo JM, Tinoco EMB, Westergaard J, Lopez NJ, Chung CP, Poulsen K, Kilian M. Racial tropism of highly toxic clone of *Actinobacillus actinomycetemcomitans* associated with juvenile periodontitis. *J Clin Microbiol* 1997;35:3037 - 3042

- Abstract -

The Frequency of Detecting *Prevotella intermedia* and *Prevotella nigrescens* in Korean Adult Periodontitis Patients

Seung - Yup Peck, Young Ku, In - Cheol Rhyu, Byung - Do Hahm

Soo - Boo Han, Sang - Mook Choi, Chong - Pyoung Chung

Department of Periodontology, College of Dentistry, Seoul National University

Prevotella intermedia has been implicated as a potent pathogen in many kinds of periodontal, pulpal and periapical diseases. However, it has been isolated from periodontally healthy adults and from edentulous children as well.

The intraspecies heterogeneity of *Prevotella intermedia* has been demonstrated in early studies and finally Shah & Gharbia confirmed the existence of 2 DNA homology groups and proposed dividing *Prevotella intermedia* into 2 species, *Prevotella intermedia* and *Prevotella*

nigrescens.

This study was designed to examine the frequency of *Prevotella intermedia* and *Prevotella nigrescens* in diseased periodontal pockets and healthy gingival sulcus of Korean people by PCR based on 16s ribosomal DNA sequence.

One hundred adults who had adult periodontitis but not taken any periodontal treatment or antibiotics during previous 6 months and 50 adults who had healthy periodontal tissue were selected for this study.

The sulcular fluid was collected into VMGA by sterilized paper point and diluted to 1,000 times in anaerobic chamber. 100 μ l of sample was cultured in 37 $^{\circ}$ C for 10 days. Among the bacterial colonies, BPB were selected and cultured in BHI broth and then *Prevotella intermedia* was identified through Gram staining and biochemical test. Identified *Prevotella intermedia* was cultured again and centrifuged. DNA was extracted from the pellet using several reagents. PCR was performed by previously designed primer.

The results were followed.

1. BPB were isolated from 39 of 100 samples of diseased periodontal pockets(39%).
2. *Prevotella intermedia* was identified from 24 of 39 BPB samples.
3. Among 24 *Prevotella intermedia*, 21 were confirmed as *Prevotella intermedia*(87.5) and 2 were confirmed as *Prevotella nigrescens*(8.33%).
4. BPB were isolated from 9 of 50 samples of periodontally healthy patients. Among them only two were

identified as *Prevotella intermedia*, that is, one was confirmed as *Prevotella intermedia* and the other was *Prevotella nigrescens*.

Key words : *Prevotella intermedia*,
Prevotella nigrescens, Polymerase chain
reaction