



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>          | +       | +       | +       | +         | +       | -      |

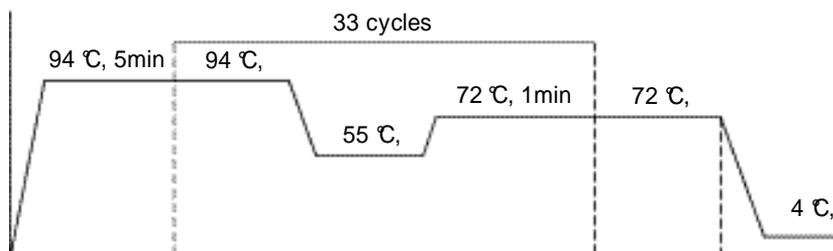


Figure 1. PCR procedure

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%  
, EtOH TE(pH 8.0)  
#35 paper point DNA 4°C  
30 가 DNA UV - spectrophotometer  
, 2ml VMGA III primer  
10% H<sub>2</sub>, 10% CO<sub>2</sub>, 80% N<sub>2</sub> design<sup>18)</sup> 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  
*Prevotella intermedia* sample 39 sample BPB가  
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 | <i>P.intermedia</i> detecting samples | <i>P.nigrescens</i> detecting samples |
|----------------------------|---------------|-----------------------|---------------------------------------|---------------------------------------|
| Infeted periodontal tissue | 100           | 39                    | 21                                    | 2                                     |
| Normal periodontal tissue  | 50            | 9                     | 1                                     | 1                                     |

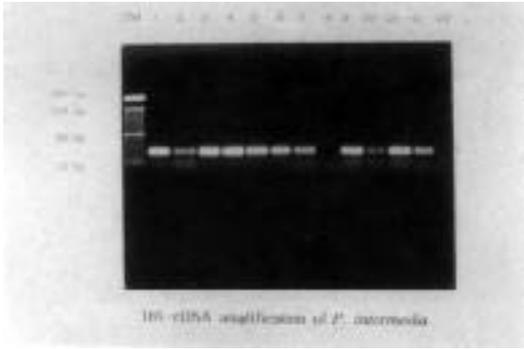


Figure 2. 16S - rDNA amplification of *Prevotella intermedia*

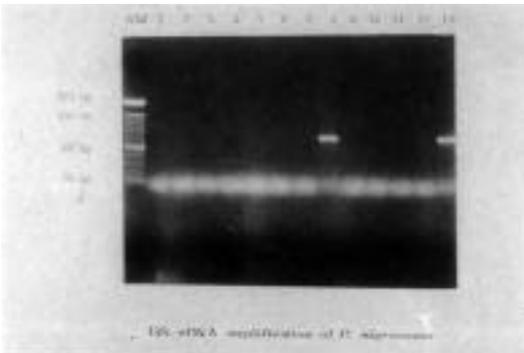


Figure 3. 16S - rDNA amplification of *Prevotella nigrescens*

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*)<sup>19)</sup>. *Porphyromonas* species 6 *Prevotella* species protoporphyrin protoheme pigment .<sup>20)</sup>

*Prevotella intermedia* gram - negative,

obligately anaerobic rod

Actinobacillus actinomycetemcomitans  
Porphyromonas gingivalis  
Prevotella intermedia

Fusobacterium nucleatum

Prevotella intermedia

<sup>21)</sup>

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 .<sup>10)</sup>

Prevotella intermedia  
Porphyromonas gingivalis

(surface property) 가

, lipopolysaccharide, capsule,  
surface appendage

Prevotella nigrescens

Prevotella intermedia  
Prevotella nigrescens

Prevotella intermedia surface  
appendage가 가 type

가 <sup>22)</sup>, negative staining

Prevotella intermedia

fibriae - like projection

Prevotella nigrescens

EM Prevotella nigrescens  
capsular layer가 Prevotella intermedia

<sup>11)</sup>. Prevotella inter -  
media Prevotella nigrescens

가

Prevotella intermedia Prevotella  
nigrescens transmission

, 가 trans -  
mission

<sup>23,24)</sup>.

Prevotella intermedia Prevotella  
nigrescens site specificity

1994 Gharbia

Prevotella intermedia

Prevotella nigrescens

nigrescens가

Prevotella intermedia가

(70.7%) <sup>11)</sup>, 1997

Prevotella intermedia

Prevotella nigrescens

Prevotella nigrescens

(73.2%)

<sup>25)</sup>. 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.

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Prevotella intermedia  
Prevotella nigrescens

, 6

가

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- Abstract -

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

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*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 $\mu$ 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*.

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