

Lactobacillus acidophilus V - 20 Porphyromonas gingivalis

I.

A. actino -

omycetemcomitans

Streptococcus sanguis (S. sanguis)

Streptococcus uberis (S. uberis)

In vitro

strep -

tococci가

A. actino -

mycetemcomitans

^{3,4)} Actinobacillus

^{1,2)} black pigmented bac -
teroides Porphyromonas gingivalis (P.
gingivalis)
가 , ,
actinomycetemcomitans(A. actino -
mycetemcomitans)

70%

¹⁹⁾ S. sanguis

^{20 - 21)}

16 -

가

5 - 7)

가

Lactobacillus

acidophilus V - 20 (L. acidophilus V - 20)

8 - 12)

가

가

가

¹⁷⁾

13 - 24)

¹⁷⁾ In vitro

L. acidophilus V -

20

In vivo

가

1982 Hillman Socransky¹³⁾

A. actinomycetemcomitans P. gin -

givalis

Plaque Index²⁵⁾

L e Silness Gingival Index²⁵⁾

16)

2

#35 paper point ,
Gracey curette
0.1Mℓ

가

L. acidophilus V - 20

glass bead 3

2Mℓ

Moller's VMGA III transport medium

60

Rogosa

(Difco, Detroit, MI, USA)

P. gingivalis

37 , 5%

3

II.

2.

L. acidophilus V - 20

1. L. acidophilus V - 20

6

6

4 - 5mm

6

10

2

2

L.

acidophilus V - 20

1

1

3

1

1

4

L. acidophilus V - 20

0.5mm

20Mℓ(2x10⁸/ml)

가

1

1

(N=3)

2Mℓ(2x10⁸/ml)

L. acidophilus V - 20

(135

5

)

MRS

L. acidophilus V - 20 10%

1

2

7

37

가

24

7

1

가 10%가

24

(Figure 1).

1

()

Periopaper strip (Pro Flow Inc.,
Amityville, New York, USA)

10

1/2

20

4

(Figure 1).

Periotron 6000 (Pro Flow Inc.,
Amityville, New York, USA)

Silness L e

	week - 2	week 0 (baseline)	day 1	day 2	day 3	day 4	day 5	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10
Gargling	scaling & root planing	gargling start	daily gargling									gargling stop					
		clinical (PI, GI) & microbiological examination	weekly clinical & microbiological examination														
Intra - pocket irrigation	& root planing	irrigation start	daily irrigation				weekly irrigation					irrigation stop					
		scaling PD, GI) & microbiological examination	clinical (PI, GCF, Lactobacillus count					clinical & microbiological examination									

Figure 1. Experimental Design

Index²⁵⁾ - Silness L e Plaque Index²⁵⁾ - L e Silness Gingival

- #35 paper point 2 3. In vitro

30 1) P. gingivalis
glass bead가 L. acidophilus V - 20

2Ml Moller's VMGA III

transport medium

60 P. gingivalis

PBS 10 10³ gingivalis 5µg/Ml hemin 5µg/Ml vitamin
Rogosa 가 가 3.7Ml MRS

37 , 5% 3 0.3Ml가 24

valis P. gingi -
37 ,

VMGA III 18 P. gingivalis 10, 10³, 10⁵,
10 10⁴ 10⁷ P. gingivalis 5%

5% , hemin, vitamin K가 5µg/Ml hemin 5µg/Ml vitamin K가
가 7 가 , Rogosa

P. gingivalis 7 , 3

0.5mm 2) beaker
wire test . 0.1M 3 - [N -

Morpholine]propanesulfonic acid(MOPS) 37 , 5%
 buffer 가 pH 7 M17SY 3%
 (M17 5% 0.5% H₂O₂ 가 (+)
 가) 20Mℓ 0.1M MOPS buffer . Carbohydrate fermentation
 가 pH 7 MRSSY test phenol red base 가 가
 (MRS 5% 0.5% 가 1%가 가
 가) 20Mℓ Streptococcus paraffin
 mutans(S. mutans) 0.1Mℓ 37 , 5%
 (2x10⁸) 1:1 0.016 inch 24 - 48
 wire (Ormco, Glendora CA, U.S.A) 50mg (+)
 가 3 beaker .
 S. mutans
 . 37 , 5% 가 4.
 24 , 3
 wire

L. acidophilus V - 20

3)

7 P. gingivalis .

Gram stain, catalase test, carbohydrate
 fermentation test ³⁶⁾.
 Catalase test MRS

Wilcoxon Signed Ranks Test
 L. acidophilus V - 20
 P. gingivalis

Table 1. The change of clinical parameter following mouth gargling with fermented milk by L.aci -
 dophilus V - 20 for 10 weeks(N=7)

Clinical recording	Baseline	week 10
Plaque Index	1.20 ± 0.50	1.00 ± 0.30
Gingval Index	0.30 ± 0.00	0.30 ± 0.01

Table 2. The change in the number of L.actobacillus(log CFU) recovered from each site following
 mouth gargling with fermented milk by L.acidophilus V - 20 for 10 weeks

	baseline	week 1	week 2	week 4	week 6	week 7	week 8	week 10
Buccal cheek	0.0 ± 0.0	0.0 ± 0.0	1.46 ± 0.95	1.90 ± 0.87	1.62 ± 0.49	1.84 ± 0.24	0.0 ± 0.0	0.0 ± 0.0
Buccal supragingival plaque on upper first molar	0.0 ± 0.0	0.26 ± 0.58	1.70 ± 1.07	1.92 ± 0.60	2.02 ± 0.60	2.26 ± 0.42	0.0 ± 0.0	0.0 ± 0.0
Buccal supragingival plaque on lower first molar	0.0 ± 0.0	0.24 ± 0.52	1.89 ± 1.25	2.59 ± 0.50	2.08 ± 0.25	2.38 ± 0.39	0.26 ± 0.5	0.0 ± 0.0
Unstimulated saliva	0.0 ± 0.0	1.98 ± 0.04	4.27 ± 0.76	4.26 ± 0.64	3.40 ± 0.93	3.34 ± 0.39	1.56 ± 1.11	0.0 ± 0.0

*: Statistically significant difference at P<0.05 by Wilcoxon test.

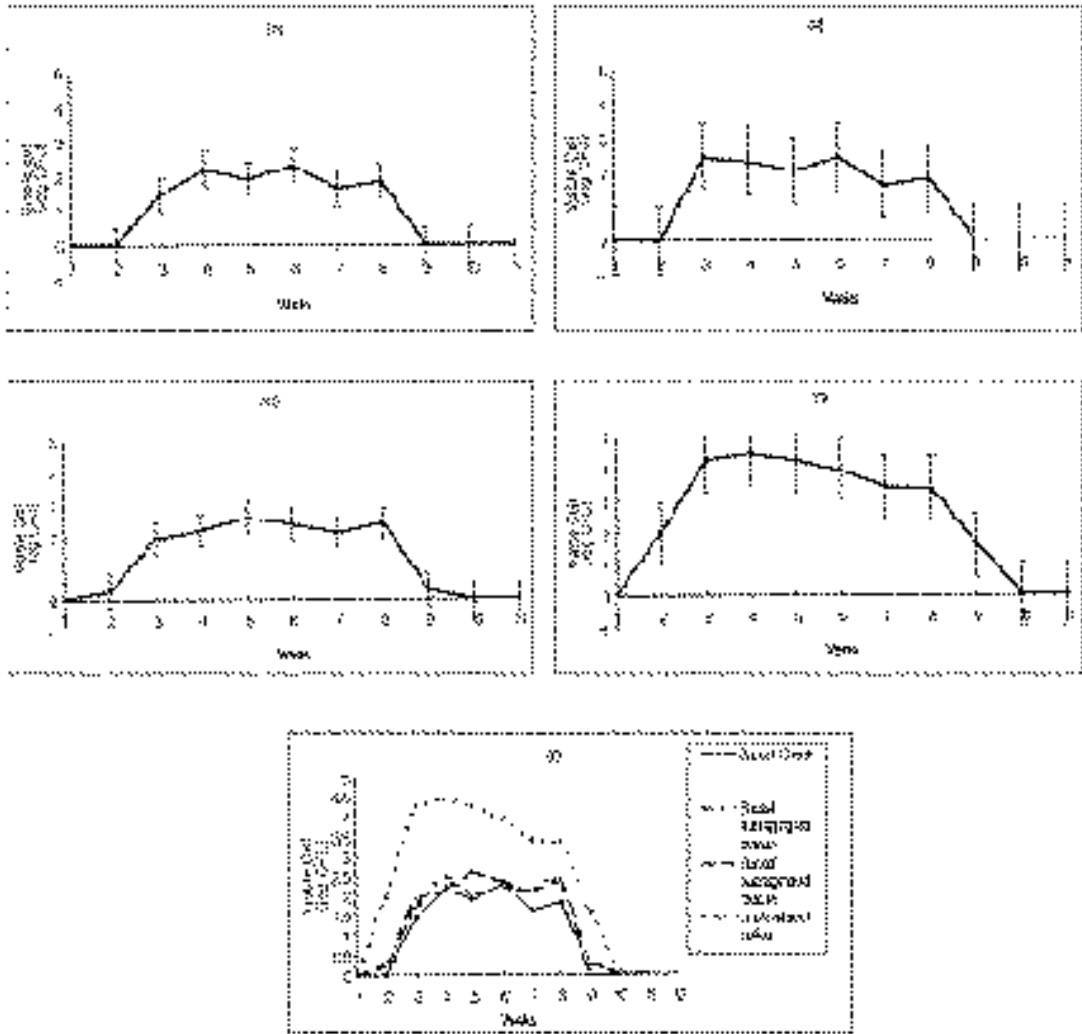


Figure 2. The change in the number of Lactobacillus (log CFU) recovered following mouth gargling with fermented milk by *L. acidophilus* V - 20 (20Mℓ of 2×10^8 /Mℓ):10 weeks study.

- (A) Buccal cheek
- (B) Supragingival plaque on the upper first molar
- (C) Supragingival plaque on the lower first molar
- (D) Unstimulated saliva
- (E) Different pattern for Lactobacillus count according to the sampling sites

Mann - Whitney Test
 P<0.05

1. *L. acidophilus* V - 20

III.

Table 3. The change of clinical parameters at baseline and after pocket irrigation with fermented milk by *L. acidophilus* V - 20 for 7 weeks(N=6)

Clinical recording	Baseline	Week 7
Plaque Index	1.00 ± 0.00	0.70 ± 0.52
Gingival crevicular fluid	50.00 ± 0.41	32.00 ± 5.82*
Probing depth	5.00 ± 1.26	3.90 ± 1.63*
Gingival Index	1.20 ± 0.41	0.70 ± 0.52

Mean ± standard deviation(N:site number)

* : Statistically significant difference between baseline and week 7 at P<0.05 by Wilcoxon test.

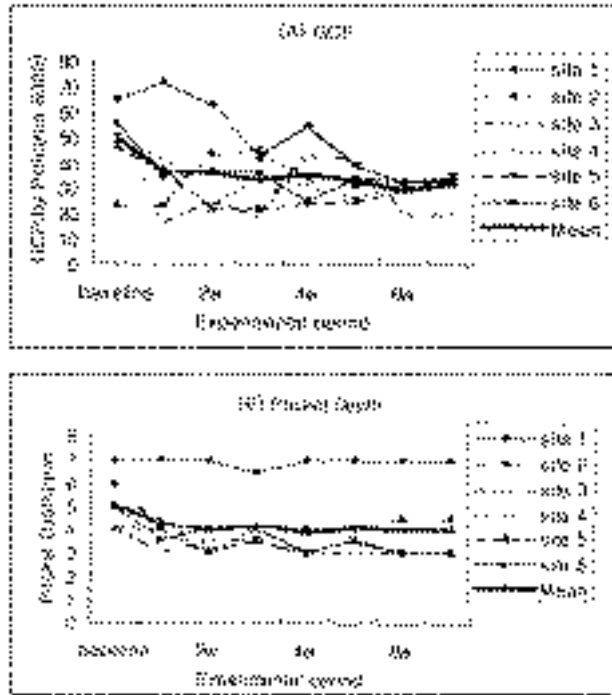


Figure 3. The change of clinical parameters following periodontal pocket irrigation with fermented milk by *L. acidophilus* V - 20 (2Ml of 2x10⁸/Ml): 7 weeks study.

(A) Gingival crevicular fluid

(B) Pocket depth(mm)

가 (Table 1). L. acidophilus V - 20 4 2 가 L. aci -
 1 - 2 가 4 dophilus V - 20 가 8
 6 - 7 가 9 가
 가 8 2 가

Table 4. The change in the number (log CFU) of Lactobacillus and P. gingivalis recovered from periodontal pocket following pocket irrigation with fermented milk by L. acidophilus V - 20 (2Mℓ of 2x10⁸/Mℓ for each pocket)

	baseline	day 0	day 1	day 3	day 5	week 1
Lactobacillus	0.56 ± 0.87	2.56 ± 0.11	0.22 ± 0.53	0.75 ± 0.83	1.74 ± 0.92	2.34 ± 0.36
P. gingivalis	4.19 ± 0.60					3.69 ± 0.46
Total viable cell	5.39 ± 0.77					5.56 ± 0.48
	week 2	week 3	week 4	week 5	week 6	week 7
Lactobacillus	3.24 ± 0.70	2.85 ± 0.80	3.07 ± 0.89	3.27 ± 1.20	2.22 ± 1.21	0.0 ± 0.0
P. gingivalis	3.12 ± 0.33	3.20 ± 0.60	3.59 ± 0.51	2.86 ± 0.76	3.30 ± 0.50	3.99 ± 0.67
Total viable cell	5.68 ± 0.59	5.42 ± 0.71	5.81 ± 0.22	5.31 ± 0.78	5.90 ± 0.38	5.58 ± 0.39

* : Statistically significant difference at the value of P<0.05 by Mann - Whitney test

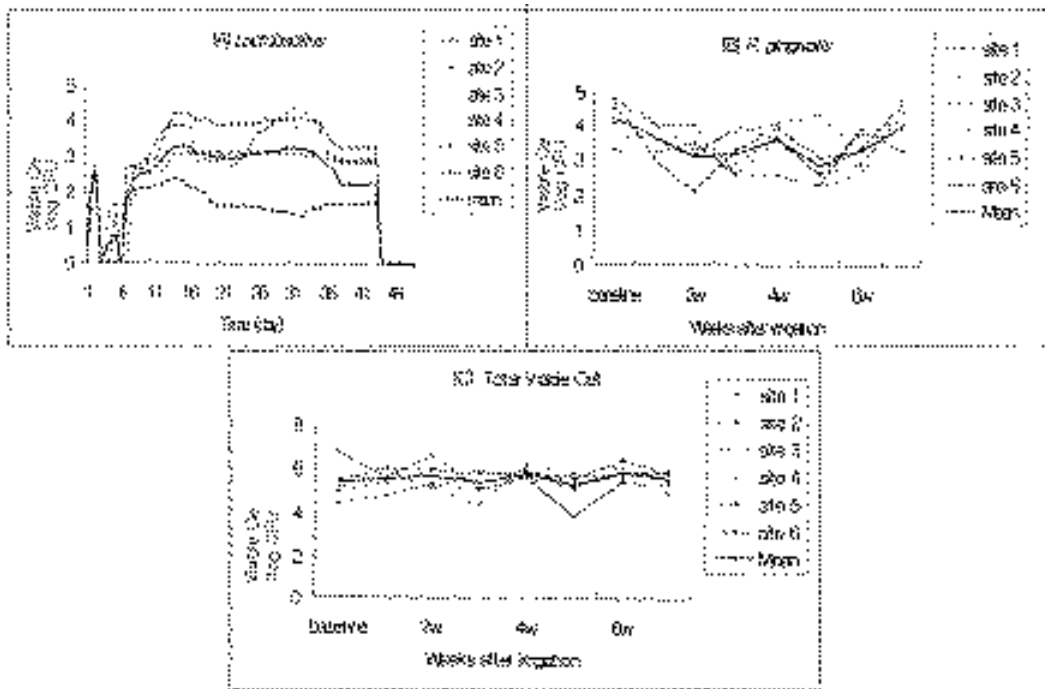


Figure 4. The change in the number of Lactobacilli and P. gingivalis recovered from periodontal pocket following pocket irrigation fermented milk by L. acidophilus V - 20 (2Mℓ of 2x10⁸/Mℓ for each pocket) : 7 weeks study.

- (A) Pattern of Lactobacillus recovery
- (B) Pattern of P. gingivalis recovery
- (C) Total anaerobic viable cell count

가 4 8
 (Table 2, Figure 2). 4
 가

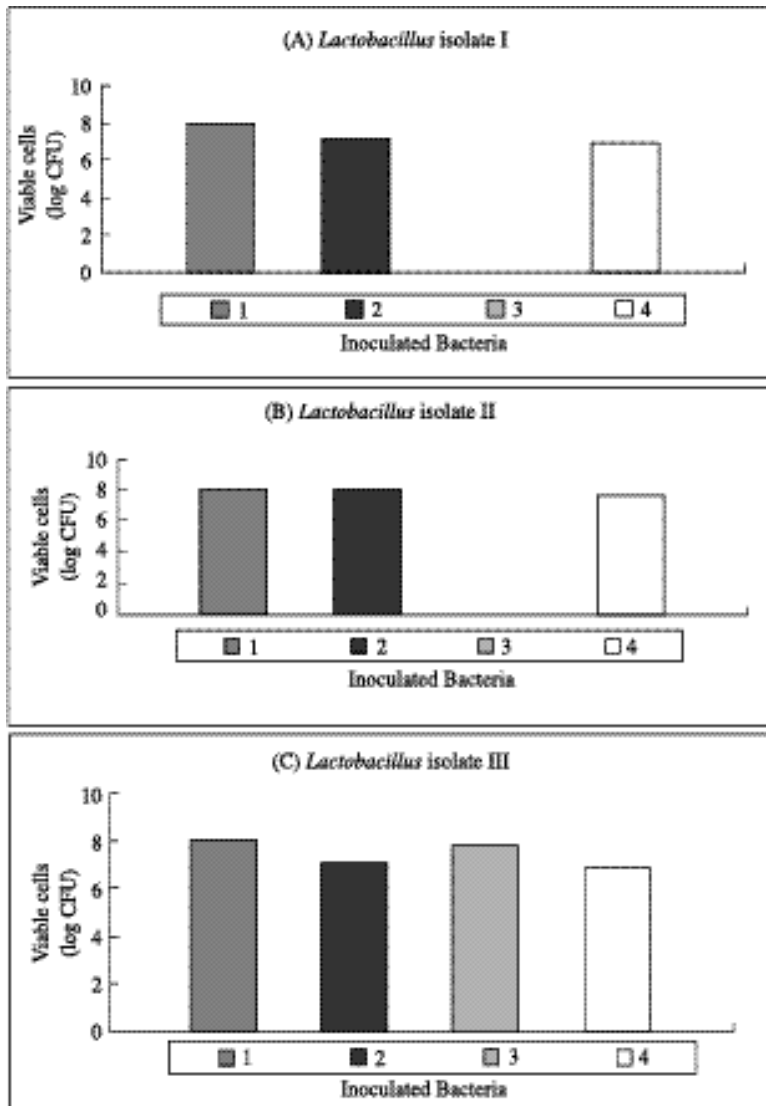


Figure 5. In vitro study for interaction between *Lactobacillus* isolates and *P. gingivalis*

- (A) *Lactobacillus* isolate I + *P. gingivalis*;
 1 : *P. gingivalis* only, 2 : *Lactobacillus* isolate I only,
 3 : *P. gingivalis*(mixed with *Lactobacillus* isolate I),
 4 : *Lactobacillus* isolate I(mixed with *P. gingivalis*)
- (B) *Lactobacillus* isolate II + *P. gingivalis*;
 1 : *P. gingivalis* only, 2 : *Lactobacillus* isolate II only,
 3 : *P. gingivalis*(mixed with *Lactobacillus* isolate II),
 4 : *Lactobacillus* isolate II(mixed with *P. gingivalis*)
- (C) *Lactobacillus* isolate III + *P. gingivalis*;
 1 : *P. gingivalis* only, 2 : *Lactobacillus* isolate III only,
 3 : *P. gingivalis*(mixed with *Lactobacillus* isolate III),
 4 : *Lactobacillus* isolate III(mixed with *P. gingivalis*)

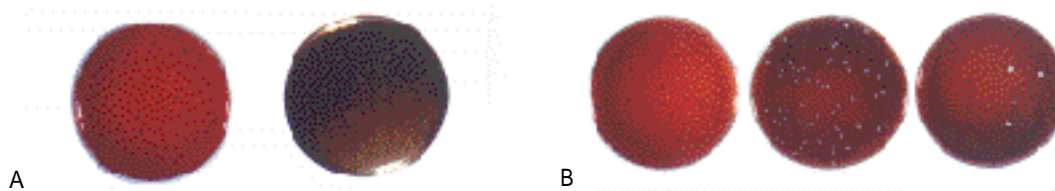


Figure 6. (A) Effect of Lactobacillus isolate I and II on the replication of *P. gingivalis*. Left: *P. gingivalis* only ($\times 10^7$), Center: Mixed culture of *P. gingivalis* and Lactobacillus isolate I ($\times 10^5$), Right: Mixed culture of *P. gingivalis* and Lactobacillus isolate II. ($\times 10^7$). Bacterial counting was done on blood agar plate containing $5\mu\text{g}/\text{Ml}$ hemin and $5\mu\text{g}/\text{Ml}$ vitamin K.

(B) Effect of Lactobacillus isolate III on the replication of *P. gingivalis*. Left: Mixed culture of *P. gingivalis* and Lactobacillus isolate III ($\times 10^7$). Bacterial counting was done on blood agar plate containing $5\mu\text{g}/\text{Ml}$ hemin and $5\mu\text{g}/\text{Ml}$

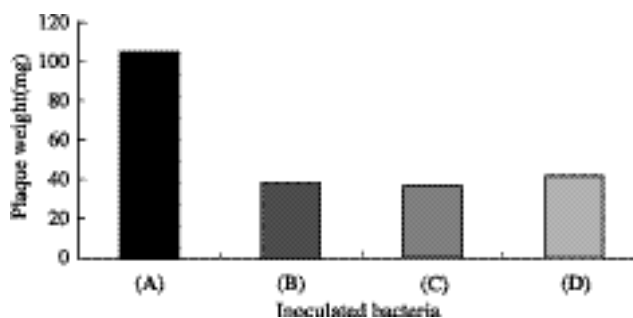


Figure 7. Plaque inhibition by Lactobacillus isolates recovered during experiment

- (A) Control - *S. mutans* only
- (B) Lactobacillus isolate I + *S. mutans*
- (C) Lactobacillus isolate II + *S. mutans*
- (D) Lactobacillus isolate III + *S. mutans*

2.	L. acidophilus V - 20	가	2	가
	<i>P. gingivalis</i>	7	<i>P. gingivalis</i>	1
		가		
6	L. acidophilus V - 20		가	7
			가	

($P < 0.05$) (Table 3, Figure 3). (Table 4, Figure 4).

3. In Vitro

가 1 가 5 가 1

Table 5. Identification of Lactobacillus strains isolated from oral cavity and periodontal pocket following mouth gargling and pocket irrigation with fermented milk by *L. acidophilus* V - 20

	Isolate I	Isolate II	Isolate III
Colony diameter	1mm	1.5mm	2mm
morphology	round	round	flat
Gram stain	+	+	+
Morphology	short rod	short rod	rod
Catalase	-	-	-
Growth at 15 °C	+	+	+
Growth at 45 °C	-	-	-
Amygdalin	-	+	-
Cellobiose	-	+	-
Fructose	+	+	+
Galactose	+	+	-
Glucose	+	+	+
Lactose	-	-	-
Maltose	+	+	-
Mannitol	-	+	-
Mannose	+	+	-
Melezitose	-	+	-
Melibiose	-	-	-
Raffinose	-	-	-
Rhamnose	-	-	-
Ribose	+	+	-
Sorbitol	-	+	-
Sucrose	+	+	-
Trehalose	-	+	-
Result	<i>L. alimentarius</i>	<i>L. casei</i> subspecies <i>casei</i>	<i>L. fructosus</i>

3 L. acidophilus V - 20 가 3 I, II P. gingivalis III P. gingivalis (Figure 5, Figure 6).

1mm 가 I, Beaker wire test I 63%, II 1.5mm II, 2mm III 67%, III 55% (Figure 7).

givalis P. gin - 3) Gram stain, catalase test, carbohydrate fermentation test I Lactobacillus alimentarius, II Lactobacillus casei subspecies casei, III Lactobacillus fructosus (Table 1) P. gingivalis 가

5).

IV.

A. actinomycetemcomitans

black pigmented bac -
teroides Porphyromonas gingivalis

S. sanguis S. uberis ,
A. actinomycetemcomitans
23,24)

P. gingivalis

, Westergren 14)

S. sanguis

S. sanguis

5-7). Hirschfeld 33), Haffajee 34)

5

S. sanguis

가

A. actinomycetemcomitans
bacteriocin

, A.
actinomycetemcomitans, P. gingivalis, P.
intermedia, B. forsythus, F. nucleatum, W.
recta

S. sanguis

14,31)

S. sanguis

20,21)

가

가

가

가

(effector strain)

, bacteriocin

30).

13-25).

16) 1982 Hillman

Socransky¹³⁾가

가

가

1

26) V - 20
 29) 가 P. gingivalis
 4 2 1
 L. acidophilus V - 20
 1 - 2
 가
 27) 17) 가 4 가 8
 L. acidophilus V - 20 6 - 7 10
 In vitro
 L. acidophilus V - 20 A. actino - 가 가 가
 mycetemcomitans P. gingivalis
 A. actino - 가
 mycetemcomitans P. gingivalis L. aci -
 dophilus V - 20
 L. acidophilus V - 20 A. L. acidophilus V - 20 가
 actinomycetemcomitans P. gingivalis 가
 L. casei
 A. actinomycetemcomitans P.
 gingivalis 가
 L. acidophilus V - 20 L. acidophilus V - 20 2 6
 4 2
 가 4mm 7
 A. actinomycetemcomi -
 tans black pigmented bacteroides
 1 가
 1 6
 pH, 가 3
 35) 가 1
 가 L. acidophilus V - 20 1 가
 가 가 가

. P. gingivalis
 1, 5 6, 6 7
 가 가 가 가 가
 L. acidophilus V - 20
 1 - 2
 3
 L. acidophilus
 V - 20
 가
 P. gingivalis
 In vitro
 P. gingivalis
 2
 P. gingivalis
 1
 P. gingivalis
 Gram stain, catalase
 test, carbohydrate fermentation test
 L. alimentarius, L. casei
 subspecies casei, L. fructosus
 P. gingivalis
 L. alimentarius L. acidophilus V - valis
 20
 , L. casei subspecies casei L.
 fructosus
 L. casei subspecies casei가 P. gingi -
 valis 가
 beaker wire test
 가 3
 55 - 63% 가
 L. acidophilus V - 20
 가 가
 가
 37 - 38) 가
 L. acidophilus V - 20
 가 가
 가
 가
 P. gingi -
 L. acidophilus V - 20
 P. gingivalis
 L. acidophilus V - 20
 P. gingivalis
 가
 Gram stain, catalase test, carbohydrate
 fermentation test
 1. L. acidophilus V - 20
 4 10

1 - 2
 , 6 - 7
 가가
 가 8
 9
 가
 가
 가
 2
 가
 가
 8
 2. L. acidophilus V - 20
 가
 1
 가 가
 1 4 1 1
 2
 3
 P. gingivalis
 가 가 7
 가
 (P<0.05)
 3. L. acidophilus V - 20
 3
 가
 P. gin -
 givalis 2
 P. gingivalis
 1
 P. gin -
 givalis 3
 55 - 63%
 4. L.
 alimentarius, L. casei subspecies casei,
 L. fructosus
 가 L. alimeatarius

L. acidophilus V - 20
 L. acidophilus V - 20
 가 P. gingivalis
 L. acidophilus V - 20
 L. acidophilus V - 20
 가
 가
 V.
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- Abstract -

The Change Pattern of Lactobacilli and Porphyromonas Gingivalis after Oral Administration of Lactobacillus Acidophilus V - 20

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National University

The treatment and prevention of periodontitis is focused on the reduction and the elimination of pathogenic bacteria, especially *A. actinomycetemcomitans* and black pigmented bacteria such as *P. gingivalis*. To prevent recurrent disease, the recolonization of these bacteria should be inhibited in the periodontal pocket. Since the replacement therapy was introduced in periodontics by Hillman et al, Jeong et al reported that hydrogen peroxide - producing *Lactobacillus acidophilus* V - 20 completely inhibited *P. gingivalis* and *A. actinomycetemcomitans* in vitro and mouth gargling with *Lactobacillus acidophilus* V - 20 in periodontitis patients during the maintenance phase improved clinical condition and reduced the No. of *P. gingivalis* and *A. actinomycetemcomitans* at 4 weeks of treatment. Prior to replacement therapy with bacteria, dynamics of microbial colonization should be considered.

This study was performed to evaluate the change in the viable cell number of Lactobacilli and *P. gingivalis* after oral administration of *L. acidophilus* V - 20. In periodontal health, gargling increased the No. of Lactobacilli in saliva, buccal mucosa, supragingival plaque from the first week, which maintained for 2 - 3 weeks after gargling stop, and then returned to the undetectable baseline level at the ninth week. In the periodontal pocket of moderate periodontitis patients, daily irrigation for 1 week and weekly irrigation for subsequent 3 weeks decreased the viable cell number of *P. gingivalis* during the period of irrigation

and increased the number of Lactobacilli, which was maintained from the second to the seventh week. *L. acidophilus* V - 20 was isolated for the first 2 weeks of oral administration, and the 3 different strains of Lactobacilli were isolated continuously for remaining period and identified as *L. alimentarius*, *L. casei* subspecies *casei* and *L. fructosus*. The first two Lactobacilli strains completely inhibited *P. gingivalis* in vitro and all the isolated Lactobacilli strains reduced the artificial plaque formation by 55 - 63%.

These results showed that mouth gargling or pocket irrigation with *L. acidophilus* V - 20 increased the No. of intraoral Lactobacilli and caused to decrease in the No. of *P. gingivalis*. This suggests that the replacement therapy by these Lactobacilli might be useful in the maintenance care of periodontal patients.