PEDIATRIC DENTAL JOURNAL 17(1): 93–99, 2007

Case Report

Clinical and microbiological evaluations of mandibular lateral incisor with radicular-gingival groove

Kazuhiko Nakano, Eriko Miyamoto, Hirotoshi Nemoto, Ryota Nomura, Seikou Shintani and Takashi Ooshima

Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry
1-8 Yamada-oka, Suita, Osaka 565-0871, JAPAN

Abstract We present a case of radicular-gingival groove identified in the mandibular left lateral incisor. A female visited our clinic at the age of 11Y5M complaining of repeated gingival inflammation. An intraoral examination found severe gingival swelling in the affected region, while clinical examinations revealed a groove from the cingulum to the apex of the mandibular left lateral incisor. A gingivectomy and professional brushing instruction were performed, with follow-up examinations given periodically. After a long interval, the patient returned at the age of 18Y4M and reported that gingival inflammation had repeatedly occurred. Subgingival dental plaque samples were collected from the affected area as well as areas around 3 normal teeth with a periodontal healthy condition, along with a saliva sample. Using bacterial DNA extracted from each sample, detection of 10 putative periodontopathic bacterial species was done by PCR, which identified *Tannerella forsythensis*, *Campylobacter rectus*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, and *Capnocytophaga sputigena* in the sample from the affected region. Further, broad-range PCR targeting 16S rRNA with direct sequencing of the samples showed a variety of bacterial species, including *Neisseria*, *Corynebacterium*, and *Fusobacterium*. *Streptococcus* species occurred at a high rate in the plaque samples from the control teeth, whereas there were none in the affected region. Our findings indicate that the bacterial profile in the area of a radicular-gingival groove may be different from other periodontal healthy sites, which might be related to the occurrence of repeated inflammation in the groove area.

Key words
16S rRNA
Parapulpal line
PCR
Periodontitis
Radicular-gingival groove

Introduction

A radicular-gingival groove is an anatomical anomaly of the teeth and has a prevalence ranging from 2.3% to 4.6%, with the maxillary lateral incisors regarded as the area with most frequent occurrence[1–3]. Such a groove is sometimes found as a radiolucent line in radiographic examinations and its main feature has been described as a "parapulpal line," which is similar to the line produced by a vertical tooth fracture[4–8]. The chief complaint regarding the lesion caused by the groove is gingival swelling and pain, and root canal treatment or a flap operation are typically selected as general treatment modalities for severe cases, and the prognosis of the lesion is considered to be poor[4–10].

We previously reported an adolescent female (11Y5M) with a radicular-gingival groove identified in the mandibular left lateral incisor along with severe gingival inflammation, which was considered to be derived from the groove[11]. A gingivectomy was carried out, followed by local irrigation and thorough instructions regarding tooth brushing. No microbiological analyses of samples from the lesion area were performed at that time. Thereafter, the patient received several periodical follow-up examinations. After a long interval between examinations,

Received on August 21, 2006
Accepted on December 6, 2006

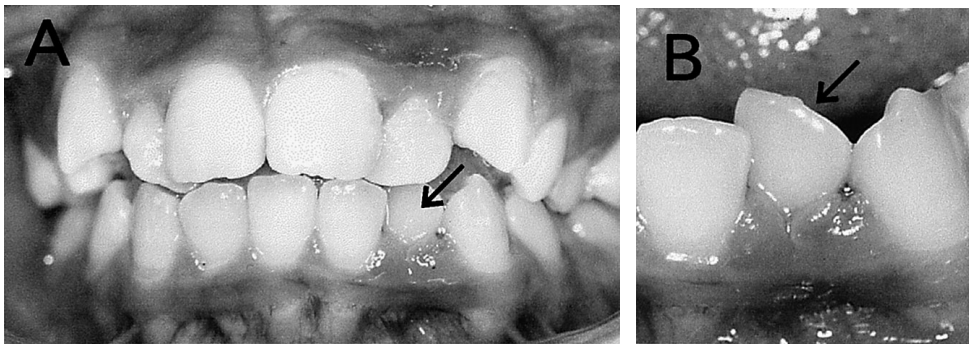

Fig. 1 Oral photographs taken at 18Y4M
(A) Frontal view of the affected area and (B) magnification of the same. Arrows indicate the affected tooth.

the patient returned to our clinic at the age of 18Y4M, and reported repeated slight swelling that had occurred for several years, though without severe signs or symptoms. Herein, we report the bacterial profiles in the lesion and unaffected areas of this patient.

Recent developments in molecular techniques has enabled prompt identification of targeted bacterial species in clinical specimens, and significantly improved specificity and sensitivity. Polymerase chain reaction (PCR) methods using primers constructed with a species-specific nucleotide alignment are widely used for the detection of specific species. In addition, a broad-range eubacterial PCR assay with amplification of bacterial DNA and subsequent direct sequencing is considered to be a reliable diagnostic tool, and has been applied for identification of bacterial profiles in clinical specimens[12,13]. Such techniques were used in the present study to analyze the bacterial profiles of the radicular-gingival groove area and other unaffected areas in the present patient.

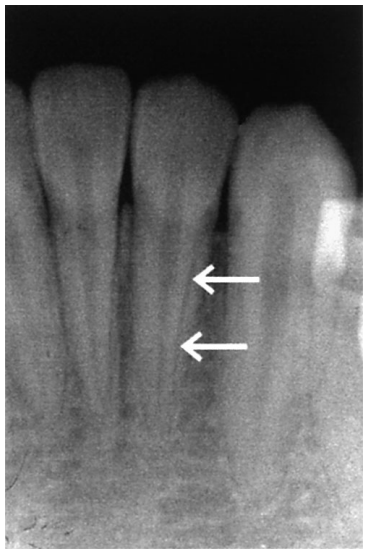

Fig. 2 Periapical radiograph taken at 18Y4M
Arrows indicate the parapulpal line.

Case Report

Case description

Previously, we presented a case of an 11-year-5-month-old girl with intensive gingival swelling due to a radicular-gingival groove[11]. Briefly, gingival swelling was localized in the distal gingiva of the mandibular left lateral incisor. Probing on the surface of the root revealed the presence of a developmental groove, while a radiographic examination showed a vertical radiolucent line from the cingulum to the apex of the affected teeth. After excising the gingiva under local anesthesia, the condition was diagnosed as epulis granulomatosa. During subsequent follow-up examinations every 4 months, gingival swelling was occasionally identified.

In March 2006, that patient returned to our clinic after a long interval for evaluation of the gingival condition. An intraoral examination revealed slight gingival swelling in the area of the mandibular lateral incisor, with bleeding on probing (Fig. 1). Periapical radiograph findings showed a vertical radiolucent line from the cervical area to the root apex (Fig. 2). We decided to perform more detailed examinations using both clinical and microbiological analyses, after receiving approval from the Ethical Committee of Osaka University Graduate School of Dentistry.

Clinical examinations

Clinical parameters, including probing depth, bleeding on probing, pus discharge, tooth mobility, plaque index[14], and gingival index[15] were examined in the present patient. Periodontal pocket depths were measured to the nearest millimeter at 6 points around the circumference of each tooth (mesio-, mid-, and disto-buccal; and disto-, mid-, and mesio-lingual) from the gingival margin to the deepest probing point, using a round-ended probe tip 0.4 mm in diameter. Bleeding on probing was scored as follows; (+) immediate bleeding on probing or (−) no bleeding. Tooth mobility was scored as follows; (2) moderate mobility (1–2 mm) in a bucco-lingual direction, and (1) slight mobility (0.2–1 mm) in a bucco-lingual direction, or (0) physiological mobility within 0.2 mm. Pus discharge was scored as follows; (+) spontaneous pus discharge, or (−) no pus discharge.

Collection of the samples and DNA extraction

Subgingival plaque and saliva samples were collected, from which bacterial DNA was extracted, using a method described previously[16]. Briefly, subgingival plaque samples were collected with sterile Gracey curettes from the affected mandibular left lateral incisor as well as the opposite incisor and maxillary bilateral incisors, then suspended in 1 m*l* of sterile saline and centrifuged at 15,000 rpm for 5 min to pellet the bacterial cells. Bacterial genomic DNA was extracted from the pellet using a DNA isolation kit (Puregene, Gentra Systems, Minneapolis, MN, USA). As for the saliva sample, expectorated whole saliva was collected from the patient and mixed with Chelex 100 (Bio-Rad Laboratories, Hercules, CA, USA), then incubated at 56°C for 30 min, followed by boiling at 100°C for 10 min. The saliva sample was then centrifuged at 15,000 rpm for 20 min and the supernatant was used as a template for PCR assays.

Detection of periodontopathic bacterial species

Microbiological examinations were performed using PCR assays, according to the procedures reported previously[16]. Ten species of Gram-negative anaerobic bacteria, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Campylobacter rectus*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, and *Treponema denticola*, were selected based on a list of putative periodontal pathogens[17,18]. The species-specific PCR primers selected for use in this study have been described previously[16]. A ubiquitous primer set that matches almost all bacterial 16S rRNA genes was also used as a positive control. PCR amplification was performed in a reaction mixture containing PCR beads (Ready-To-Go; Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Identification of bacterial species

Identification of bacterial species in each sample was performed using a broad-range PCR technique targeting 16S rRNA with direct sequencing, as described previously[13]. Briefly, 16S rRNA was amplified by PCR with the broad-range 16S rRNA primers 536f (5′-CAG CAG CCG CGG TAA TAC-3′) and 1050r (5′-CAC GAG CTG ACG ACA-3′)[12], after which the PCR products were separated by electrophoresis on a 1.5% agar gel and the amplified DNA fragments were extracted using a QIAEX II gel Extraction Kit (QIAGEN Sciences, Düsseldorf, Germany). Extracted DNA was directly cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA), then 40 clones from the affected region and saliva sample, as well as 30 clones from the dental plaque samples from 3 unaffected regions were randomly chosen, and the sequences of the amplified fragments were determined. To identify the bacterial species, the 16S rRNA sequences were compared with those available in the GenBank, EMBL, and DDBJ databases using the gapped BLASTN 2.0.5 program obtained from the National Center for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/BLAST/). Identification at the genus level was defined as a 16S rRNA sequence similarity of 97% with that of the GenBank prototype strain sequence.

Results

C. sputigena, *A. actinomycetemcomitans*, *T. forsythensis*, *C. rectus*, and *E. corrodens* were detected in the subgingival plaque samples from the affected lesion where inflammation was identified, in which the maximum periodontal pocket was 4 mm and bleeding on probing was identified (Table 1). The subgingival plaque sample from the maxillary left lateral incisor was shown to contain *P. nigrescens* in addition to the 5 species identified in the sample from the affected area. On the other hand, only a

Table 1 Site-specific clinical gingival conditions and detection of periodontal bacteria

	Dental plaque				Saliva
	LL2	LR2	UL2	UR2	
P. gingivalis	−	−	−	−	+
T. denticola	−	−	−	−	−
C. ochracea	−	−	−	−	−
C. sputigena	+	−	+	+	+
P. intermedia	−	−	−	−	−
P. nigrescens	−	−	+	−	−
A. actinomycetemcomitans	+	−	+	−	+
T. forsythensis	+	−	+	−	−
C. rectus	+	−	+	−	+
E. corrodens	+	−	+	−	−
Periodontal condition					
Pocket depth (mm)	4	2	2	3	
Bleeding on probing	+	−	−	−	
Gingival index	1	0	1	1	
Plaque index	1	1	2	1	
Mobility	1	0	0	0	
Pus exudate	−	−	−	−	

LL2: mandibular left lateral incisor, LR2: mandibular right lateral incisor,
UL2: maxillary left lateral incisor, UR2: maxillary right lateral incisor

single species of *C. sputigena* was detected in the maxillary right lateral incisor region and none of the 10 periodontopathic bacteria were detected in the mandibular right lateral incisor region. As for the saliva sample, *P. gingivalis*, *C. sputigena*, *A. actinomycetemcomitans*, and *C. rectus* were identified.

Broad-range PCR and sequencing analyses revealed a variety of bacterial species in the dental plaque samples from the 4 regions as well as the saliva sample (Table 2). In the plaque sample from the affected lesion, *Kingella* species were the most frequently detected (25%), followed by *Neisseria* (17.5%), *Corynebacterium* (15%), *Fusobacterium* (7.5%), and *Oribacterium* (7.5%). The major difference between the species identified was that *Streptococcus* species were more frequently detected in the control location (16.7–26.7%) than in the affected area (0%) ($P<0.05$; Fisher's exact probability test). The saliva sample contained a variety of bacterial species, similar to the profile of the plaque samples.

Discussion

There have been several case reports of a radicular-gingival groove regarding evaluations of the prognoses[4–10]. The subject ages in those cases ranged from 12 to 45 years of age and the maximum periodontal pocket depths were between 6 and 9 mm, which were regarded as severe. Root canal treatments or flap operations were performed, and the prognosis evaluated for a range of 6 months to 3 years. Extraction of the affected teeth was reported in most of the cases in a range of 6 months to 3 years, while no significant recurrent signs or symptoms were observed for 1.5 to 3 years in several of the cases.

In the present patient, severe gingival swelling and inflammation were observed at the first visit 7 years prior. However, a gingivectomy procedure and professional tooth brushing instruction led to lesion stability for the following period. In contrast, recently reported clinical examinations after a long interval have found inflammation localized in the affected region. Based on the report of our patient, it is estimated that local inflammation recurred during the period when she did not visit our clinic. In this study, we evaluated the bacterial profile in the affected region and compared it to other periodontal healthy regions in the same patient.

Table 2 Site-specific bacterial profiles analyzed by broad-range PCR and sequencing

Dental plaque				Saliva (n = 40)
LL2 (n = 40)	LR2 (n = 30)	UL2 (n = 30)	UR2 (n = 30)	
Kingella (25%)	*Actinomyces* (43%)	*Neisseria* (27%)	*Neisseria* (37%)	*Neisseria* (35%)
Neisseria (18%)	*Streptococcus* (20%)	*Streptococcus* (27%)	*Actinomyces* (20%)	*Haemophilus* (20%)
Corynebacterium (15%)	*Lautropia* (13%)	*Corynebacterium* (10%)	*Streptococcus* (17%)	*Prevotella* (8%)
Fusobacterium (8%)	*Haemophilus* (7%)	*Oribaclum* (7%)	*Corynebacterium* (13%)	*Corynebacterium* (5%)
Oribaclum (8%)	*Veillonella* (3%)	*Eubacterium* (3%)	*Kingella* (7%)	*Veillonella* (5%)
Cardiobacterium (5%)	*Cardiobacterium* (3%)	*Fusobacterium* (3%)	*Fusobacterium* (3%)	*Capnocytophaga* (5%)
Selemonas (5%)	*Kingella* (3%)	*Haemophilus* (3%)	No matching (3%)	*Abiotrophia* (3%)
Actinomyces (3%)	*Neisseria* (3%)	*Selemonas* (3%)		*Lautropia* (3%)
Campylobacter (3%)	No matching (3%)	*Veillonella* (3%)		*Pseudomonas* (3%)
Capnocytophaga (3%)		*Prevotella* (3%)		*Streptococcus* (3%)
Propionibacterium (3%)		No matching (10%)		No matching (13%)
No matching (8%)				

LL2: mandibular left lateral incisor, LR2: mandibular right lateral incisor,
UL2: maxillary left lateral incisor, UR2: maxillary right lateral incisor

The 10 periodontal bacterial species were selected based on previous reports regarding their association with periodontitis clinically, which analyzed the distribution of those species in periodontal healthy children as well as those with periodontal diseases[19–22]. In recent studies, the bacterial group known as the red complex species, composed of *P. gingivalis*, *T. forsythensis*, and *T. denticola*, has been shown to be highly associated with the severity of periodontitis[23]. In Japan, an analysis of 6 periodontal species in 95 children (8–11 years old) and 107 adolescents aged 15 years old revealed that *P. gingivalis* and *T. forsythensis*, and *T. forsythensis* and *C. rectus*, respectively, were markers of an increased risk of periodontitis onset[24,25]. In the present case, *T. forsythensis* and *C. rectus* were detected the subgingival plaque samples collected from the affected area (Table 1), indicting an increased risk of periodontitis. However, 1 of the 3 dental plaque samples taken from unaffected regions also showed the presence of those 2 species, though the clinical parameters for that control region did not show severe gingival inflammation, in contrast to the region of the tooth with a radicular-gingival groove. Thus, it is possible that other species associated with the inflammation were present.

The bacterial profile of the dental plaque sample from the affected lesion determined by species-specific sets of primers was different from that shown by the use of broad-range primers designed for the common alignment of 16S rRNA genes in a variety of eubacterial species (Tables 1 and 2). Of the 10 putative periodontopathic bacterial species analyzed with the species-specific sets of primers, very few were identified using the present broad-range PCR and sequencing method. We previously analyzed the bacterial profiles of dental plaque samples taken from 32 subjects (aged 45 to 84 years) using the same broad-range PCR method in the present study, which showed that *Streptococcus* species (20.7%)

were the most prevalent, followed by *Capnocytophaga* (11.4%), *Prevotella* (8.3%), *Fusobacterium* (8.0%), *Neisseria* (7.9%), *Porphyromonas* (2.9%), *Actinomyces* (2.6%) and *Tannerella* (1.4%)[13]. Those results indicate that most of the bacterial species listed in Table 1 could have been detected if they had been present. Therefore, it is possible that the amounts of 10 tested periodontal bacterial species were actually low in the dental plaque samples analyzed, even though several species showed positive reactions to the species-specific sets of primers.

Several studies have focused on the identification of species in normal bacterial flora and periodontitis lesions using broad-range PCR and sequencing methods. *Neisseria* and *Kingella* species (known as *Neisseria*-like species), isolated more often from healthy subjects than periodontitis patients[26], were widely identified in all the present samples, indicating a low possibility that they were associated with the lesion caused by the groove. *Streptococcus* species were frequently detected in plaque samples collected from the 3 control sites, however, none of the 40 clones from the affected area were *Streptococcus* species. Since *Streptococcus* species are known to be one of the members of the bacterial community in healthy subjects, in contrast to those with periodontitis[26,27], it is reasonable to speculate that the environmental changes in the periodontal pocket close to the groove were conductive to the establishment of anaerobic bacterial species.

Broad-range PCR methods are introduced as valuable tools for clinical diagnoses in a variety of medical fields[12]. One of the interesting clinical applications of broad-range PCR and sequencing is for analysis of periodontitis, as described in the report of Sakamoto *et al.*[28], in which bacterial profiles before and after periodontal treatment were compared. Molecular approaches such as PCR with species-specific sets of primers and the broad-range methods may be able to reveal indicator species related to the occurrence of the periodontitis and estimation of its prognosis. Accumulation of such data in future studies will be useful.

Acknowledgments

This study was supported by a part of the 21st Century COE program entitled "Origination of Frontier BioDentistry" at Osaka University Graduate School of Dentistry supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Everett, F.G. and Kramer, G.M.: The disto-lingual groove in the maxillary lateral incisor; A periodontal hazard. *J Periodontol* **43**: 352–361, 1972.
2) Withers, J.A., Brunsvold, M.A., Killoy, W.J. and Rahe, A.J.: The relationship of palato-gingival grooves to localized periodontal disease. *J Periodontol* **52**: 41–44, 1981.
3) Kogon, S.L.: The prevalence, location and conformation of palato-radicular grooves in maxillary incisors. *J Periodontol* **57**: 231–234, 1986.
4) Peikoff, M.D., Perry, J.B. and Chapnick, L.A.: Endodontic failure attributable to a complex radicular lingual groove. *J Endod* **11**: 573–577, 1985.
5) Robison, S.F. and Cooley, R.L.: Palatogingival groove lesions: recognition and treatment. *Gen Dent* **36**: 340–342, 1988.
6) Mayne, J.R. and Martin, I.G.: The palatal radicular groove. Two case reports. *Aust Dent J* **35**: 277–281, 1990.
7) Goon, W.W., Carpenter, W.M., Brace, N.M. and Ahlfeld, R.J.: Complex facial radicular groove in a maxillary lateral incisor. *J Endod* **17**: 244–248, 1991.
8) Cecilia, M.S., Lara, V.S. and de Moraes, I.G.: The palato-gingival groove. A case of failure in root canal treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **85**: 94–98, 2001.
9) Meister, F. Jr., Keating, K., Gerstein, H. and Mayer, J.C.: Successful treatment of a radicular lingual groove: Case report. *J Endod* **9**: 561–564, 1983.
10) Andreana, S.: A combined approach for treatment of developmental groove associated periodontal defect. A case report. *J Periodontol* **69**: 601–607, 2001.
11) Nakano, K., Zhu, J., Kawaguchi, M., Ooshima, T. and Sobue, S.: A case of radicular-gingival groove identified in the mandibular lateral incisor. *Jpn J Ped Dent* **38**: 237–241, 2000. (in Japanese)
12) Rovery, C., Greub, G., Lepidi, H., Casalta, J.P., Habib, G., Collart, F. and Raoult, D.: PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. *J Clin Microbiol* **43**: 163–167, 2005.
13) Nakano, K., Inaba, H., Nomura, R., Nemoto, H., Takeda, M., Yoshioka, H., Matsue, H., Takahashi, T., Taniguchi, K., Amano, A. and Ooshima, T.: Detection of cariogenic *Streptococcus mutans* in extirpated heart valve and atheromatous plaque specimens. *J Clin Microbiol* **44**: 3313–3317, 2006.
14) Silness, J. and Löe, H.: Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* **22**: 121–135, 1964.
15) Löe, H. and Silness, J.: Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* **21**: 533–551, 1963.

16) Tamura, K., Nakano, K., Hayashibara, T., Nomura, R., Fujita, K., Shintani, S. and Ooshima, T.: Distribution of 10 periodontal bacteria in saliva samples from Japanese children and their mothers. *Arch Oral Biol* **51**: 371–377, 2006.
17) Darveau, R.P., Tanner, A. and Page, R.C.: The microbial challenge in periodontitis. *Periodontol 2000* **14**: 12–32, 1997.
18) Zambon, J.J.: Microbiology of periodontal disease. *In:* Contemporary Periodontics. (Genco, R.J., Goldman, H.M. and Cohen, D.W. eds.) The CV Mosby Company, St. Loius, 1990, pp. 147–160.
19) Ooshima, T., Nishiyama, N., Hou, B., Tamura, K., Amano, A., Kusumoto, A. and Kimura, S.: Occurrence of periodontal bacteria in healthy children: a 2-year longitudinal study. *Community Dent Oral Epidemiol* **31**: 417–425, 2003.
20) Nakano, K., Nishiyama, N., Tamura, K., Sasaki, H. and Ooshima, T.: Clinical and microbiological evaluations of gingival fibromatosis in children: Report of two cases. *Ped Dent J* **14**: 141–146, 2004.
21) Nakano, K., Tamura, K., Ogawa, T., Kawabata, K. and Ooshima, T.: Oral findings and microbiological evaluation in a case of triple-X syndrome. *Ped Dent J* **15**: 219–225, 2005.
22) Tamura, K., Nakano, K., Miyake, S., Takada, A. and Ooshima, T.: Clinical and microbiological evaluations of acute periodontitis in areas of teeth applied with orthodontic bands. *Ped Dent J* **15**: 212–218, 2005.
23) Rocas, I.N., Siqueira, J.F. Jr., Santos, K.R. and Coelho, A.M.: "Red Complex" (*Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*) in endodontic infections: a molecular approach. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **91**: 468–471, 2001.
24) Suda, R., Kurihara, C., Kurihara, M., Sato, T., Lai, C.H. and Hasegawa, K.: Determination of eight selected periodontal pathogens in the subgingival plaque of maxillary first molars in Japanese school children aged 8–11 years. *J Periodontal Res* **38**: 28–35, 2003.
25) Suda, R., Kobayashi, M., Nanba, R., Iwamaru, M., Hayashi, Y., Lai, C.H. and Hasegawa, K.: Possible periodontal pathogens associated with clinical symptoms of periodontal disease in Japanese high school students. *J Periodontol* **75**: 1084–1089, 2004.
26) Kumar, P.S., Griffen, A.L., Moeschberger, M.L. and Leys, E.J.: Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. *J Clin Microbiol* **43**: 3944–3955, 2005.
27) Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I. and Dewhirst, F.E.: Defining the normal bacterial flora of the cavity. *J Clin Microbiol* **43**: 5721–5732, 2005.
28) Sakamoto, M., Huang, Y., Ohnishi, M., Umeda, M., Ishikawa, I. and Benno, Y.: Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes. *J Med Microbiol* **53**: 563–571, 2004.