Detection of *Porphyromonas gingivalis* from Saliva by PCR by Using a Simple Sample-Processing Method

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Simple sample-processing methods for PCR detection of *Porphyromonas gingivalis*, a major pathogen causing adult periodontitis, from saliva were studied. The ability to detect *P. gingivalis* from 118 saliva samples by PCR after boiling and Chelex 100 processing was compared with bacterial culture. *P. gingivalis* was detected three times more often by PCR than by culture. Chelex 100 processing of saliva proved to be effective in preventing PCR inhibition and was applied to determine the occurrence of *P. gingivalis* in saliva samples from 263 Finnish subjects between 5 and 80 years of age. The occurrence of *P. gingivalis* increased with age, and it was detected by PCR in the saliva of 5.0% of subjects between 5 and 10 years of age, 13.8% of subjects between 11 and 20 years of age, 13.4% of subjects between 21 and 30 years of age, and 63.3% of subjects between 31 and 80 years of age. The results indicate that *P. gingivalis* is a rare finding in saliva from periodontally healthy children and young adults but a frequent one in saliva from adult periodontitis patients.

*Porphyromonas gingivalis*, a black-pigmented gram-negative anaerobic rod, is a major pathogen causing adult periodontitis (18). *P. gingivalis* is frequently isolated from subgingival plaque of periodontitis patients, whereas it can be cultured only occasionally from periodontally healthy adults and is usually not isolated from children (4, 8, 9, 13, 15, 25).

Saliva is the most probable vehicle for person-to-person transmission of oral bacteria. Thus, it is likely that the presence of *P. gingivalis* in saliva is a prerequisite for its transmission. Saliva represents an easily and noninvasively obtainable sample containing bacteria from all oral sites, e.g., the mucosa and supra- and subgingival plaque. Furthermore, it is also rather easily obtainable from the oral cavities of young children. However, the proportion of shed periodontal bacteria in the saliva microbiota is relatively low, a fact that makes bacterial detection of periodontal bacteria from saliva samples from periodontally healthy children and young adults but a frequent one in saliva from adult periodontitis patients.

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**Materials and Methods**

**Subjects, sampling, and bacterial culture.** The material used in this study consisted of saliva samples from 263 periodontally healthy or diseased subjects (age range, 5 to 80 years). The periodontal status of a subject was defined as healthy when no signs of periodontal breakdown were found. Periodontitis was diagnosed when the presence of periodontal breakdown was verified in a clinical and/or radiological examination. Periodontitis was further classified according to guidelines of the American Academy of Periodontology (1).

A subset of 118 samples was used for evaluation of the PCR method. The occurrence of *P. gingivalis* in Finnish subjects as determined by PCR was studied using all 263 saliva samples. The samples were collected during several previous studies, between 1985 and 1996, by using paraffin chewing stimulation and preserved at −70°C until used in the present study. For the detection of *P. gingivalis* by bacterial culture, 142 of the 263 saliva samples had been serially diluted immediately after sampling and cultured on *Brucella* agar (BBI Microbiology Systems, Cockeysville, Md.) supplemented with lysed horse blood (5%), hemin (5 μg/ml), and vitamin K1 (10 μg/ml). The plates were incubated anaerobically in jars filled by the evacuation-replacement method with a mixture of gases (85% N2, 10% H2, 5% CO2). The isolates were identified as *P. gingivalis* on the basis of having the typical colony color and morphology, lacking colony autofluorescence, having positive trypsin-like enzyme activity (19), and having a positive indole reaction.

**Sample processing for PCR.** Two rapid methods of sample processing for PCR were tested.

(i) **Boiling.** A 30-μl aliquot of each of the 118 saliva specimens was boiled for 10 min and then centrifuged at 10,000 × g for 5 min, and 5 μl (or 0.5 μl) of the supernatant was used as a template for PCR.

(ii) **Chelex 100 treatment.** A 50-μl aliquot of each of the 263 samples of saliva was incubated with 12.5 μl of 25% (wt/vol) Chelex 100 (Bio-Rad Laboratories, Hercules, Calif.), a cation-chelating resin, at 56°C for 30 min before being boiled and centrifuged as described above. A 6-μl aliquot of the supernatant was then used as a template for PCR.

**PCR amplification.** Two *P. gingivalis*-specific primers described by Slots et al. (21) were used to amplify a 404-bp fragment of the 16S rRNA gene: primer 1 (5′-AGG CAG CCT GCC ATG CG-3′) and primer 2 (5′-ACT GTT AGC AAC TAC CGA TGT-3′). The specificity of the PCR method was investigated by using purified DNA from 26 clinical *P. gingivalis* isolates from unrelated subjects and that from 34 isolates of 30 species genera other than *P. gingivalis* as templates for PCR. The latter included *Porphyromonas asaccharolytica* ATCC 9344 and...
There was some discrepancy between the results obtained after sample boiling and those obtained after Chelex 100 processing (Table 2). Of the 118 samples, 28 (23.7%) were P. gingivalis-positive and 69 (58.5%) were P. gingivalis-negative by both methods, whereas 21 (17.8%) samples repeatedly gave discrepant results.

### TABLE 1. Detection of P. gingivalis in saliva samples from 118 subjects by bacterial culture and PCR

<table>
<thead>
<tr>
<th>PCR result</th>
<th>Culture result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive (%)</td>
<td>No. negative (%)</td>
<td></td>
</tr>
<tr>
<td>After boiling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>7 (5.9)</td>
<td>33 (28.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>4 (3.4)</td>
<td>74 (63.8)</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>107</td>
</tr>
</tbody>
</table>

| After Chelex 100 treatment |               |       |
| Positive | 11 (9.3) | 26 (22.0) | 37 |
| Negative | 0 | 81 (68.6) | 81 |
| Total | 11 | 107 | 118 |

### Inhibitory effect of saliva on PCR.

Of the 118 saliva samples processed by the boiling method, 23 (19.5%) showed a distinct inhibition of PCR amplification when 50 P. gingivalis cells were added to each 50 μl PCR mixture. Dilution of the saliva decreased the inhibitory effect, since all but 3 of the 23 inhibitory samples showed good amplification when 0.5 μl instead of 5 μl of saliva (in both cases with 50 P. gingivalis cells) was used as a template for PCR. Although dilution resulted in amplification of most of the 23 salivary samples, the simultaneously occurring 10-fold rise in the detection level is not desirable. When Chelex 100 was applied to process the 23 samples that showed inhibition after boiling, 18 samples gave distinct amplicons and 5 samples gave weak amplicons. The detection limit of the PCR after boiling and after Chelex 100 processing of the sample was one P. gingivalis cell per PCR in water. The detection limit in saliva showing no PCR inhibition was also one cell per PCR. However, the amplification signal obtained with a few cells (1 to 5 cells per PCR) was constantly weaker in saliva than in water (Fig. 1).

### Occurrence of P. gingivalis.

Since Chelex 100 processing of the saliva samples decreased PCR inhibition most effectively, it was used to investigate the occurrence of P. gingivalis in 263 Finnish subjects from 5 to 80 years of age (Table 3). The saliva samples from 142 of these 263 subjects were also cultured for the presence of P. gingivalis (Table 3). The occurrence of P. gingivalis increased with age, since the organism was detected by PCR in 3 (5.0%) of the 60 subjects between 5 and 10 years of age, in 12 (13.8%) of the 87 subjects between 11 and 20 years of age, in 9 (13.4%) of the 67 subjects between 21 and 30 years of age, and in 31 (63.3%) of the 49 subjects between 31 and 80 years of age. P. gingivalis was not found by bacterial culture in any of the 9 subjects between 5 and 10 years of age, whereas it was isolated from 3 (6.1%) of the 49 subjects between 11 and 20 years of age, from 1 (2.3%) of the 44 subjects...
between 21 and 30 years of age, and from 7 (17.5%) of the 40 subjects between 31 and 80 years of age (Table 3).

Data on periodontal status were available for 254 subjects. *P. gingivalis* was detected by PCR in 19 (10.3%) of the 185 periodontally healthy subjects; in 6 (21%) of the 28 subjects with prepubertal, localized juvenile periodontitis or some other type of early-onset periodontitis; and in 28 (70%) of the 40 subjects with adult periodontitis. The corresponding figures obtained by bacterial culture were 2 (2.6%) of 78, 1 (3.8%) of 26, and 7 (22.6%) of 31 subjects (Table 3).

**DISCUSSION**

In the present study, 20% of the saliva samples showed PCR inhibition when the boiling method was used for sample processing. PCR inhibition of other biological samples (e.g., sputum, stool, and genital ulcer specimens) is well known, and different methods (e.g., immunomagnetic separation, phenol-chloroform extraction, and use of capture resins) have been suggested for the inactivation of PCR inhibitors (2, 10, 12). However, many of these other methods are laborious and expensive. In the present study, a simple sample-processing technique involving the use of Chelex 100 resin prior to PCR amplification proved to be very applicable for the detection of *P. gingivalis* in salivary samples. In the Chelex 100 processing method, only one reagent was added to the sample and the processing was performed in a single tube, which minimizes the number of steps involving handling and hence the risk of contamination. The applicability of Chelex 100 for inactivating PCR inhibitors in saliva probably stems from the ability of Chelex 100 to chelate divalent ions. Chelex 100 has previously been shown to enhance the efficiency of DNA extraction, especially from gram-positive and acid-fast bacteria, and to protect DNA at high temperatures (6).

In the present study, some discrepancies in PCR detection of *P. gingivalis* from saliva were observed when the two sample preparation methods were compared. Why some samples were *P. gingivalis*-positive by PCR after being subjected to Chelex 100 processing but *P. gingivalis*-negative by PCR after being boiled can be explained by the better ability of the Chelex 100 method to abolish PCR inhibition by saliva. The existence of samples that were *P. gingivalis*-positive by bacterial culture but *P. gingivalis*-negative by PCR after being boiled supports this suggestion. The reason why some samples were *P. gingivalis*-negative by PCR after being subjected to Chelex 100 processing but *P. gingivalis*-positive by PCR after being boiled remained unknown. The results were reproducible, and the finding can be explained neither by differences in the sensitivities of the two methods nor by the uneven distribution of very low numbers of *P. gingivalis* cells in the samples.

In the present study, *P. gingivalis* was detected in saliva samples three times more often by PCR than by bacterial culture, which is well in accordance with data from earlier PCR studies using the same primers for the detection of *P. gingivalis* in subgingival plaque samples (3, 21). Also, in other studies using primers targeted to different regions of the 16S rRNA gene or to the collagenase gene, *P. gingivalis* has been detected in subgingival samples more frequently by PCR than by bacterial culture (21, 26). The higher rate of detection by PCR is most likely due to the higher sensitivity of the PCR technique, which is especially important in studies on mixed bacterial flora. In addition, in contrast to bacterial culture, PCR amplification also detects nonviable bacterial cells present in the sample. In all earlier studies in which detection of *P. gingivalis* by PCR and detection of this bacterium by bacterial culture have been compared, the sample material has been subgingival plaque. *P. gingivalis* has been cultured more often and in higher numbers from subgingival plaque than from saliva, which is in accordance with data from earlier studies in which detection of *P. gingivalis* in saliva was compared to subgingival plaque samples using the same primers for PCR amplification of *P. gingivalis* (21). It is possible to detect *P. gingivalis* in saliva only when the concentration of the bacterium is high enough and when PCR inhibition is less severe.

**TABLE 3. Occurrence of *P. gingivalis* in Brazilian subjects with different periodontal statuses as determined by PCR after Chelex 100 processing and by bacterial culture**

<table>
<thead>
<tr>
<th>Mean age (range), in yr</th>
<th>Periodontal status</th>
<th>Detection by:</th>
<th>n</th>
<th>No. positive (%)</th>
<th>n</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6 (5–10)</td>
<td>Healthy</td>
<td>PCR</td>
<td>59</td>
<td>3 (5.1)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Prepubertal periodontitis</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>15.2 (11–20)</td>
<td>Healthy</td>
<td>PCR</td>
<td>83</td>
<td>11 (13.3)</td>
<td>45</td>
<td>2 (4.4)</td>
</tr>
<tr>
<td></td>
<td>Localized juvenile periodontitis</td>
<td>PCR</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Diabetes-associated periodontitis</td>
<td>PCR</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1 (100)</td>
</tr>
<tr>
<td>24.5 (21–30)</td>
<td>Healthy</td>
<td>PCR</td>
<td>34</td>
<td>2 (5.9)</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Localized juvenile periodontitis</td>
<td>PCR</td>
<td>16</td>
<td>2 (12.5)</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Early-onset periodontitis</td>
<td>PCR</td>
<td>8</td>
<td>4 (50.0)</td>
<td>8</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td></td>
<td>No data</td>
<td>PCR</td>
<td>9</td>
<td>1 (11.1)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>42.8 (31–80)</td>
<td>Healthy</td>
<td>PCR</td>
<td>9</td>
<td>3 (33.3)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Adult periodontitis</td>
<td>PCR</td>
<td>40</td>
<td>28 (70)</td>
<td>31</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>22.5 (5–80)</td>
<td></td>
<td>PCR</td>
<td>263</td>
<td>55 (20.9)</td>
<td>142</td>
<td>11 (7.7)</td>
</tr>
</tbody>
</table>

*FIG. 1. Detection limit of PCR after Chelex 100 processing. Lanes 1 to 6, 500, 50, 5, 2, 1, and 0 *P. gingivalis* ATCC 33277 cells in saliva per PCR, respectively; lanes 7 to 12, 500, 50, 5, 2, 1, and 0 *P. gingivalis* cells in water per PCR, respectively; M, 1-kb DNA ladder.*
proportions from subgingival plaque than from saliva (24, 25). However, due to the high detection limit for P. gingivalis in saliva, culture studies may underestimate its prevalence in saliva samples.

In this study, the occurrence of P. gingivalis as determined by PCR detection seemed to increase with age of the Finnish subjects. P. gingivalis was infrequently detected in samples from children under 10 years of age (5%), and it was only rarely detected in specimens from teenagers and young adults (13.4 to 13.8%), whereas most adults (63.3%) over 30 years of age harbored this bacterium. The low rate of detection of P. gingivalis in children is in accordance with a study by Ashimoto et al. (3) in which they detected P. gingivalis by PCR in 14% of children aged around 7 years. However, in a study by McClellan et al. (14), P. gingivalis was detected by PCR in 37% of subjects under 18 years of age, with similar frequencies irrespective of age. Differences in the PCR methodologies are a likely cause for the discrepant results, since the nested-PCR method used by McClellan et al. (14) is more sensitive than the PCR methods used in other studies. However, McClellan et al. did not report the specificity of the nested-PCR method. In culture studies, P. gingivalis has not been isolated (7–9, 13), or has been isolated only extremely rarely (4, 16, 17), from the oral cavities of children and young adults, which coincides well with the low isolation frequency of the present study. Similar to PCR studies, hybridizations with DNA probes have revealed higher rates of detection of P. gingivalis in the older age groups as well as a positive correlation between detection of P. gingivalis and increasing age (20). However, the higher frequency of detection of P. gingivalis with total chromosomal DNA probes compared with that of bacterial culture may be explained in part by the hybridization of these probes to other species, leading to false-positive results (20, 23).

While P. gingivalis was rarely detected by PCR in saliva from periodontally healthy subjects or from subjects with localized juvenile periodontitis in the present study, it was common in adult periodontitis patients. Since the prevalence of periodontitis is very low in children and adolescents but increases with age, being almost ubiquitous in middle-aged individuals (5), it is difficult to find a representative study population in which subjects of various ages would have similar periodontal statuses. In the present study, most of the subjects under 30 years of age were periodontally healthy whereas older subjects commonly exhibited adult periodontitis. The increased rates of detection of P. gingivalis in the older age groups may be related to the differences in the periodontal statuses of the subjects. Also, in previous PCR studies, P. gingivalis has rarely been detected in subjects without periodontitis (3, 22) but has frequently been found in adult periodontitis patients (3).

In conclusion, the present study shows the applicability of Chelex 100 processing of salivary samples for PCR amplification. P. gingivalis was rarely detected in saliva from periodontally healthy Finnish children and young adults. The prevalence of P. gingivalis increased with age, suggesting that oral colonization takes place mainly during adulthood.

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REFERENCES


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