SHORT COMMUNICATION

DETECTION OF MYCOPLASMA FERMENTANS IN HUMAN SALIVA WITH A POLYMERASE CHAIN REACTION-BASED ASSAY

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(Accepted 16 February 1996)

Summary—Mycoplasma fermentans and other mycoplasma species may be associated with human immunodeficiency virus infection. Little is known about the ecology of this micro-organism and its natural habitat. A polymerase chain reaction (PCR)-based assay was used to detect M. fermentans in whole saliva. The hypothesis was tested that M. fermentans is present on the mucosal surfaces of the mouth and oropharynx. Whole saliva was collected from 110 adults. The 206-bp amplification product of DNA purified from these samples was detected in ethidium bromide-stained 6% polyacrylamide gels in 49 (44.5%) samples tested. All samples were confirmed by Southern blotting with a probe based on an internal sequence of the expected amplification product. The data suggest that this organism is often found in saliva and on oropharyngeal mucosal surfaces. Saliva may play a part in its transmission between individuals. Saliva sampling may be helpful in further studies of the ecology and distribution of the micro-organism in human populations. Copyright © 1996. Elsevier Science Ltd.

Key words: Mycoplasma fermentans, polymerase chain reaction, saliva.

Mycoplasma fermentans has been proposed as a human pathogen and possible cofactor in HIV infection (Lo, 1986; Lo et al., 1989a, c; 1991). This micro-organism is found at autopsy in tissues from patients with AIDS (Bauer et al., 1991). It is also pathogenic in humans in the absence of HIV infection and causes fatal systemic infections in non-human primates (Lo et al., 1989b, c). Recently, a PCR-based assay has been utilised to detect M. fermentans in clinical samples (Hawkins et al., 1992; Wang et al., 1992; Dawson et al., 1993; Katseni et al., 1993). This assay amplifies a 206-bp fragment from an insertion sequence-like element unique to M. fermentans. Using this assay, the association between M. fermentans infection and progression of AIDS was investigated. Subsequent studies have compared the prevalence of the micro-organism in clinical samples from HIV-seropositive and -seronegative individuals. M. fermentans was detected in blood and urine samples from HIV-seropositive individuals but not in HIV-negative controls.

Little is known about the natural habitat and ecology of M. fermentans. We have now investigated the hypothesis that M. fermentans is a colonizer of human mucosal surfaces. The PCR-based assay was used to determine the prevalence of M. fermentans in whole-saliva samples from adults.

Participants (58 males and 52 females) were recruited from students and staff of the Boston University Medical Center. Ages ranged from 23 to 59 years (mean = 31.05 ± 6.59). One millilitre of whole saliva was collected in a sterile tube from each participant. DNA extraction from saliva was by the method of Cone et al., 1993. Extracted DNA samples were resuspended in 200 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8). Each PCR amplification reaction contains 10 μl of the resuspended DNA. Mycoplasma fermentans ATCC19989 was obtained from American Type Culture Collection (Rockville, MD) and grown in pre-reduced heart infusion broth (Difco, Detroit, MI) in an anaerobic Gas-Pak jar (BBL, Microbiology Systems, MD) at 37°C for 2 weeks. DNA was obtained from M. fermentans by salt–chloroform extraction. After precipitation in isopropanol and ethanol, DNA was resuspended in TE buffer. This DNA served as positive control for the PCR-based assay for M. fermentans. To preclude contamination of saliva samples with M. fermentans, handling of M. fermentans and processing of saliva

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Abbreviations: AIDS, acquired immune deficiency syndrome; dGTP, 2-deoxyguanosine-5′-triphosphate; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; dUTP, deoxyuridine triphosphate.
samples was carried out at different sites. In addition, a negative control, consisting of all the reagents except for the template DNA, was also run with each round of amplification to rule out the possibility of false positives.

*Mycoplasma fermentans*-specific DNA sequences were amplified once for each sample, as described by Wang *et al.* (1992), using a Geneamp PCR reagent kit with AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) in a thermal cycler (MiniCycler® PTC-150, MJ Research, Massachusetts). Concentrations of 0.5 μM for each primer and 10 μl of the template DNA were added. Primers RW004 (GGA CTA TTG TCT AAA CAA TTT CCC) and RW005 (GGT TAT TCG ATT TCT AAA TCG CCT) were from Hu *et al.*, 1990. Analysis of PCR amplification products was by polyacrylamide gel electrophoresis and Southern blotting (Southern, 1975). A 73-bp, internal, digoxigenin-labelled probe was generated using the PCR method with primers 5′ GAT GAG TGT ATT GTC ATCC 3′ and 5′ AAC GTA GAA GAG AAT GGC 3′ as described by Hawkins *et al.*, 1992. Digoxigenin labelling was done by replacing 70 μM of dGTP with digoxigenin–dUTP in the PCR reaction mixture. *Mycoplasma fermentans* (10 μl) served as template DNA for the labelling reaction. The reaction mixture was subject to electrophoresis on a 2%, low-melting agarose gel, and the 73-bp amplification product excised. The excised gel was boiled for 10 min and added to the hybridization

![Diagram of PCR-amplified products](image)

Fig. 1. Analysis of polymerase chain reaction (PCR)-amplified products from representative saliva samples. The PCR products were analysed by electrophoresis in a polyacrylamide gel stained with ethidium bromide (A) and by hybridization with a digoxigenin-labelled oligonucleotide probe specific for *M. fermentans* (B), following electrophoretic transfer of DNA to a nylon membrane. Lane (a) is a digoxigenin-labelled size marker; lane (b) is a negative control; lanes (c), (d), (e), (g), (h), and (i) are samples positive for *M. fermentans*; lane (f) is a sample negative for *M. fermentans*; lane (j) is a 206-bp amplified product of DNA purified from *M. fermentans* ATCC 19989. Left-hand dotted arrows correspond to DNA size standards of 300, 200 and 100 bp.
buffer. For Southern blotting, fractionated amplification product was electroblotted on a nylon membrane, denatured, fixed, rinsed and air-dried (Dawson et al., 1993). Hybridization and immunological detection were done according to manufacturer’s instructions (Genius® System; Boehringer Mannheim, Indianapolis). A positive result was determined by the presence of a 206-bp band on the membrane (see Fig. 1).

All saliva samples tested for M. fermentans were analysed by ethidium bromide staining of the polyacrylamide gel and Southern blotting. Of the 110 samples tested, 49 (44.5%) were positive for M. fermentans. Among males, 25 of 57 (43.8%) were positive, while 24 of 53 (45.3%) females were positive. The 206-bp amplification product was not detected in any of the negative controls, indicating no detectable contamination of reagents with DNA. For Southern blotting, fractionated amplification were done according to manufacturer’s instructions.

The ~2 analysis showed no significant difference in the prevalence of M. fermentans between males and females ($p > 0.05$). However, it is of interest that of the 38 Asian participants, 63.2% showed detectable M. fermentans in saliva compared with only 35.2% of the 71 Caucasians. It is possible that geographical or racial factors may predispose certain individuals to M. fermentans infection.

Our findings suggest that M. fermentans is part of the normal oral flora, as compared with other studies that have not detected this micro-organism in HIV-negative individuals. Interestingly, only the study of Katseni et al. (1993) showed similar proportions of M. fermentans infection between HIV-positive and -negative individuals. They speculated that the throat may be a primary source of M. fermentans. Our findings are consistent with theirs, and further suggest that the oral mucosa may also harbour M. fermentans. In fact, the observed prevalence of M. fermentans in our study (44.5%) higher than that reported by Katseni et al. from throat swabs of HIV-seronegative individuals (20%). Whether this reflects differences in sampling or the ecology of M. fermentans has not been resolved.

Our findings may have significance for the biology, pathogenicity and ecology of M. fermentans. These data suggest that saliva, and by extension the mouth and oropharynx, may be a significant reservoir of M. fermentans in human populations, and that it may be a benign colonizer of the oral mucosa in a healthy, young and middle-aged adult population. It pathogenicity in HIV-positive individuals and AIDS patients may be related to their immunocompromised status. Thus, it may be classified as an opportunistic pathogen in AIDS patients.

**Table 1. Detection of M. fermentans DNA in whole saliva**

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 57)</th>
<th>Females (n = 53)</th>
<th>Asians (n = 38)</th>
<th>Caucasians (n = 71)</th>
<th>Blacks (n = 1)</th>
<th>Total (n = 110)</th>
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<td>Negative</td>
<td>32/57</td>
<td>29/53</td>
<td>14/38</td>
<td>46/71</td>
<td>1</td>
<td>61/110</td>
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<td></td>
<td>56.2%</td>
<td>54.7%</td>
<td>36.8%</td>
<td>64.8%</td>
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<td>55.5%</td>
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<tr>
<td>Positive</td>
<td>25/57</td>
<td>24/53</td>
<td>24/38</td>
<td>25/71</td>
<td>0</td>
<td>49/110</td>
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<td>43.8%</td>
<td>45.3%</td>
<td>63.2%</td>
<td>35.2%</td>
<td></td>
<td>44.5%</td>
</tr>
</tbody>
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REFERENCES
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