Detection of *Helicobacter pylori* DNA in the saliva of patients complaining of halitosis

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Helicobacter pylori infection, which causes peptic ulcers and gastric cancer, is considered a possible cause of halitosis. Recently, the oral cavity was identified as a possible *H. pylori* reservoir, particularly in the presence of periodontal disease, which is a cause of halitosis. The purpose of this study was to evaluate by PCR the prevalence of oral *H. pylori* in the saliva of subjects complaining of halitosis. Samples were obtained from 326 non-dyspeptic subjects, comprising 251 subjects with actual malodour and 75 subjects without halitosis. DNA was extracted from the samples, and the presence of *H. pylori* and periodontopathic bacteria including *Porphyromonas gingivalis*, *Treponema denticola* and *Prevotella intermedia* was examined by PCR. *H. pylori* was detected in 21 (6.4%) of 326 samples. The methyl mercaptan concentration and periodontal parameters including tooth mobility, periodontal pocket depth (PPD) and occult blood in the saliva were significantly greater in the *H. pylori*-positive subjects. Each of the periodontopathic bacteria was also detected at a significantly higher frequency in the *H. pylori*-positive subjects. Among those patients with a PPD of ≥ 5 mm and a tongue coating score of ≤ 2 , no difference was observed in oral malodour levels between the *H. pylori*-positive and -negative subjects. However, the presence of occult blood in the saliva and the prevalence of *Prevotella intermedia* were significantly greater in the *H. pylori*-positive subjects. *H. pylori* was detected in 16 (15.7%) of 102 subjects with periodontitis, suggesting that progression of periodontal pocket and inflammation may favour colonization by this species and that *H. pylori* infection may be indirectly associated with oral pathological halitosis following periodontitis.

Received 21 May 2008
Accepted 5 August 2008

INTRODUCTION

Helicobacter pylori is a spiral, microaerophilic, Gram-negative bacterium that colonizes the human gastrointestinal tract, primarily the stomach (Covacci *et al.*, 1999). It is also believed to be responsible for gastritis and peptic ulcers, and is a risk factor for gastric cancer (Dunn *et al.*, 1992; Parsonnet *et al.*, 1991; Warren & Marshall, 1983); however, the mode of transmission of *H. pylori* is poorly understood. Several studies have detected *H. pylori* in the human oral cavity, particularly in patients with gingivitis or chronic periodontitis (Anand *et al.*, 2006; Gebara *et al.*, 2006; Souto & Colombo, 2008), and have thus suggested that the oral cavity is the primary extragastric reservoir for *H. pylori*. In contrast, other studies have failed to find evidence supporting the role of the oral cavity as a major reservoir of *H. pylori* (Czesnikiewicz-Guzik *et al.*, 2004; Loster *et al.*, 2006).

Halitosis is a common problem in humans, and oral malodour is largely caused by periodontitis, tongue debris, poor oral hygiene, deep caries, inadequately fitted restorations and endodontic lesions (Rosenberg *et al.*, 1991; Tonzetich, 1977; Yoneda *et al.*, 2006); specifically, it is primarily the result of the microbial metabolism of amino acids in local debris (Scully *et al.*, 1994). Many of the compounds that contribute to oral malodour are volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide (CH_3SCH_3) (Kleinberg & Westbay, 1990; Tonzetich, 1977). Periodontopathic bacteria, including *Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella intermedia* and *Fusobacterium nucleatum*, produce H_2S and CH_3SH (Fukamachi *et al.*, 2005; Tonzetich & McBride, 1981). Additionally, methylamine, dimethylamine, propionic acid, butyric acid, indole, scatole and cadaverine have been reported to cause oral malodour (Goldberg *et al.*, 1994; Kostelc *et al.*, 1981). To evaluate the level of oral malodour in patients complaining of halitosis, clinicians typically measure their VSC level and give them an organoleptic test (OLT) (Lee *et al.*, 2007).

Abbreviations: OLT, organoleptic test; PPD, periodontal pocket depth; VSC, volatile sulfur compound.

To diagnose halitosis, a simple classification with corresponding treatment needs has been developed (Miyazaki *et al.*, 1999; Yaegaki & Coil, 2000), which includes the categories of genuine halitosis, pseudo-halitosis and halitophobia. Genuine halitosis is subclassified as physiological or pathological halitosis, and pathological halitosis is subclassified as oral or non-oral pathological halitosis. Physiological halitosis is caused largely by poor oral hygiene and tongue debris, whereas oral pathological halitosis is caused largely by periodontal disease (Yaegaki & Sanada, 1992). Non-oral pathological halitosis can originate from the upper respiratory tract and from other sources that are carried by blood and exhaled in the lung (Delanghe *et al.*, 1997; Tangerman & Winkel, 2007). Gastrointestinal diseases are also generally believed to cause halitosis (Adler *et al.*, 2005; Tydd & Dyer, 1974). In this study, oral *H. pylori* DNA was detected in the saliva of subjects complaining of halitosis and the relationships between oral *H. pylori* infection and oral health and malodour were examined.

METHODS

Study population. Three hundred and twenty-six subjects (137 males and 189 females with a mean age $\pm$ SD of 45.3 ± 15.0 years) complaining of halitosis and presenting to the Oral Malodour Clinic at Fukuoka Dental College Medical and Dental Hospital, Japan, between June 2005 and February 2008 were recruited for the study. Informed consent was obtained from all enrolled individuals, none of whom had taken antibiotics within 3 months prior to the study. All of the subjects were non-dyspeptic. Prior to the appointment for malodour assessment, each subject was asked to refrain from eating, drinking, chewing, brushing or rinsing the mouth for at least 5 h. On the basis of the VSC data, 251 subjects were determined to have actual malodour (total VSCs ≥ 0.25 p.p.m.), while 75 subjects had almost no malodour beyond socially acceptable levels (total VSCs < 0.25 p.p.m.).

Malodour assessment. The severity of malodour in each individual was determined using an OLT and gas chromatography (model GC14B; Shimadzu Works). For the OLT, each patient was instructed to exhale through the mouth with moderate force into a Teflon sampling bag (GL Science) for 2–3 s to prevent dilution of the mouth odour with lung and room air. This procedure was repeated until approximately 1 litre of air was obtained. Two or three evaluators then estimated the odour at a distance of approximately 10 cm from the sampling bag. The OLT scores ranged from 0 to 5 (0, absence of odour; 1, questionable odour; 2, slight malodour; 3, moderate malodour; 4, strong malodour; 5, severe malodour) (Miyazaki *et al.*, 1999) and the mean values of the scores assigned by the three judges were recorded. For the gas chromatographic measurements, the subjects were asked to remain quiet with a closed mouth for 30 s, after which mouth air (10 ml) was aspirated with a gas-tight syringe. Subsequently, the samples were injected onto a gas chromatograph column at 70 °C. A glass column was packed with 25 % β , β 9-oxydipropionitrile on a 60–80 mesh Chromosorb W AW-DMCS-ST device (Shimadzu Works) fitted with a flame photometric detector. The concentration of each VSC was determined based on the values for standard H_2S , CH_3SH and CH_3SCH_3 gas prepared with a PD-1B permeator (GL Science). The threshold levels for genuine halitosis were defined as an OLT score of ≥ 3 , ≥ 0.15 p.p.m. H_2S in the mouth air, ≥ 0.05 p.p.m. CH_3SH in the mouth air, ≥ 0.02 p.p.m. CH_3SCH_3 in the mouth air and ≥ 0.25 p.p.m. total VSCs (Tonzetich & Ng, 1976; Yaegaki & Coil, 2000).

Clinical examinations. The oral health of each patient was evaluated based on the number of teeth, number of caries, number of fillings, no-good margin, mobility of the teeth, periodontal pocket depth (PPD), degree of tongue coating, volume of stimulated salivary flow and the presence of occult blood in the saliva. PPD was measured at six points around each tooth in all of the subjects. Each subject's periodontal status was determined based on the presence of teeth with a PPD of ≥ 5 mm. The total area and thickness of the tongue coating were determined by clinical inspection with a simple modification (Oho *et al.*, 2001; Suzuki *et al.*, 2008). The degree of tongue coating was assessed based on conventional criteria, with a simple modification (0, no tongue coating; 1, thin tongue coating covering less than one-third of the tongue dorsum; 2, thick tongue coating covering approximately one-third of the tongue dorsum or thin tongue coating covering one-third to two-thirds of the tongue dorsum; 3, thick tongue coating covering one-third to two-thirds of the tongue dorsum or thin tongue coating covering more than two-thirds of the tongue dorsum; and 4, thick tongue coating covering more than two-thirds of the tongue dorsum) (Oho *et al.*, 2001). Tongue-coating scores of ≥ 3 were considered to be a cause of oral malodour. The volume of stimulated salivary flow was measured using the chewing gum test. Each patient was asked to pool saliva in the oral cavity then spit into a vessel every minute throughout the entire collection period (5 min). Testing for the presence of occult blood in the saliva was performed using a Perioscreen (Sunstar).

Conventional PCR assays. The prevalence of *H. pylori* and major periodontal bacteria, including *Porphyromonas gingivalis*, *Treponema denticola* and *Prevotella intermedia*, in the saliva of the subjects was determined by PCR. The sequences of oligonucleotide primers used in this study are listed in Table 1. The chromosomal DNA of the bacteria in the saliva was prepared by physical rupture using a Disruptor Genie cell disrupter (Scientific Industries) with zirconium and tungsten carbide beads. Briefly, 500 μ l stimulated whole saliva and an equal amount of PBS were mixed and centrifuged at 12 000 g for 10 min. Subsequently, 300 μ l lysis buffer (50 mM Tris/HCl, pH 8.0; 1 mM EDTA; and 1 % SDS), 0.3 g zirconium beads and a tungsten carbide bead (3 mm diameter) were added to the precipitate, which was then boiled at 100 °C for 10 min. The oral bacteria in the saliva samples were ruptured using a cell disrupter at room temperature for 3 min. The DNA was then purified by repeated phenol/chloroform extraction, precipitated with 100 % ethanol and resuspended in 100 μ l TE (10 mM Tris/HCl, pH 8.0; and 1 mM EDTA). Each 10 μ l volume of the PCR mixture contained 0.25 mM deoxynucleotide triphosphates, 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 5 U *Taq* polymerase, 2 μ M each primer and 2 μ l template DNA. Amplification was conducted using a TaKaRa PCR thermal cycler with the following temperature profile: 94 °C for 2 min, followed by 30 cycles of 94 °C for 10 s, 55 °C for 15 s and 72 °C for 1 min, with a final extension at 72 °C for 5 min. The amplified products were loaded onto 1.5 % (w/v) agarose gels, separated by electrophoresis, stained with ethidium bromide (0.5 μ g ml⁻¹) and photographed under UV light.

Specificity of the PCR assay. The specificity of the PCR assay was tested against chromosomal DNA from 18 oral bacteria: *Streptococcus mutans* Xc, *Streptococcus sobrinus* 6715, *Streptococcus mitis* 903, *Streptococcus oralis* ATCC 10557, *Streptococcus sanguinis* ATCC 10556, *Streptococcus gordonii* DL1, *Streptococcus pneumoniae* WU2, *Actinomyces naeslundii* ATCC 51655, *Actinomyces viscosus* ATCC 43146, *Eikenella corrodens* 1085, *Haemophilus aphrophilus* NCTC 5908, *Aggregatibacter actinomycetemcomitans* Y4, *Porphyromonas gingivalis* ATCC 33277 and W83, *F. nucleatum* ATCC 10953, *Tannerella forsythensis* ATCC 43037, *Prevotella intermedia* ATCC 25611 and *Treponema denticola* ATCC 35405. Chromosomal DNA from *H. pylori* ATCC 43504 was used as a positive control. The PCR

Table 1. Species-specific primers for PCR used in this study

Species	Primer sequence (5'→3')*	Product size (bp)	Reference
<i>H. pylori</i>	F: CTATGACGGGTATCCGGC R: ATTCCACCTACCTCTCCCA	375	Nyan <i>et al.</i> (2004)
<i>Porphyromonas gingivalis</i>	F: TGTAGATGACTGATGGTGAAAACC R: ACGTCATCCCCACCTTCCTC	211	Yoshida <i>et al.</i> (2003)
<i>Treponema denticola</i>	F: TAATACCGAATGTGCTCATTTACAT R: TCAAAGAAGCATTCCCTCTTCTTCTTA	316	Ashimoto <i>et al.</i> (1996)
<i>Prevotella intermedia</i>	F: TTTGTTGGGGAGTAAAGCGGG R: TCAACATCTCTGTATCCTGCGT	575	Ashimoto <i>et al.</i> (1996)

*F, Forward; R, reverse.

amplicons were further identified by RFLP analysis using enzymes *MspI* and *TaqI*.

Statistical analysis. Statistical analysis of the differences in the frequency of malodour, clinical symptoms and periodontopathic bacteria between the *H. pylori*-positive and -negative specimens was performed using a χ^2 test and an unpaired *t*-test. The SPSS statistical software package was used for all analyses (release 11.0 J; SPSS Japan).

RESULTS AND DISCUSSION

Specificity of the PCR assay

A 375 bp PCR product was obtained with chromosomal DNA from a pure culture of *H. pylori* ATCC 43504. Similar patterns were obtained by digesting PCR amplicons from clinical samples and *H. pylori* ATCC 43504 using RFLP analysis (Fig. 1).

Prevalence of oral *H. pylori* in a Japanese population

The demographic and clinical parameters of the subject group are shown in Table 2. Overall, *H. pylori* was detected in 21 (6.4%) of 326 samples. The prevalence of oral *H.*

pylori as determined by PCR in Japanese gastric patients was previously reported as 7 or 26% in saliva and 24% in dental plaque (Ishihara *et al.*, 1997; Shimada *et al.*, 1994). This is the first report of oral *H. pylori* in non-dyspeptic Japanese individuals. Cross-sectional studies have consistently shown a gradual increase in *H. pylori* seroprevalence with age, which has been interpreted as a birth cohort effect reflecting a decrease in the rate of acquisition in successive generations as sanitation and the standard of living have improved (Malaty *et al.*, 2003; Parsonnet *et al.*, 1992). The highest reported prevalence of oral *H. pylori* was observed in subjects between 30 and 39 years (10.3%) and between 50 and 59 years in the present study (9.2%). No significant difference was detected between the *H. pylori*-positive and -negative subjects according to age, sex, number of teeth, number of caries, number of fillings or stimulated salivary flow. Based on the results of serological tests to detect the presence of antibodies against *H. pylori*, the carriage rate of *H. pylori* is reported to be 20–80% for adults in the developed world and more than 90% in the developing world (Taylor & Blaser, 1991). The prevalence of *H. pylori* in Japanese adults is reported to be 55% (Fujisawa *et al.*, 1999). Souto & Colombo (2008) detected *H. pylori* in the subgingival biofilm samples of 24% of the population without dyspepsia. Conversely, Olivier *et al.* (2006) did not detect *H. pylori* in dental samples from subjects with positive stomach biopsies. These findings indicate that the oral cavity may act as a reservoir for *H. pylori*, regardless of infection in the stomach.

Prevalence of oral *H. pylori* and oral malodour

The CH₃SH levels as measured by gas chromatography were significantly higher in the *H. pylori*-positive group than in the *H. pylori*-negative group; however, no significant difference was observed in the levels of H₂S, CH₃SCH₃ and total VSCs, or in the OLT score (Table 3). CH₃SH gas is thought to be elevated in patients with periodontal involvement (Yaegaki & Sanada, 1992). Among the clinical symptoms associated with oral malodour, periodontal symptoms including tooth mobility, a PPD of ≥5 mm, a PPD of ≥6 mm and occult blood

Fig. 1. PCR products of clinical specimens and chromosomal DNA of *H. pylori* ATCC 43504 analysed by RFLP analysis. Lanes 1 and 6, *H. pylori*-positive saliva sample from patient no. 61; lanes 2 and 7, *H. pylori*-positive saliva sample from patient no. 72; lanes 3 and 8, *H. pylori*-positive saliva sample from patient no. 128; lanes 4 and 9, *H. pylori*-positive saliva sample from patient no. 241; lanes 5 and 10, chromosomal DNA of *H. pylori* ATCC 43504. In lanes 1–5, the fragments were digested with *MspI*, whilst in lanes 6–10, the fragments were digested with *TaqI*.

Table 2. Demographic and clinical parameters for the *H. pylori*-positive and -negative subjects

Parameter	<i>H. pylori</i> -positive (n=21)	<i>H. pylori</i> -negative (n=305)
Age (years)	43.8 ± 12.1	45.4 ± 14.9
10–19 (%)	0.0 (0/21)	5.9 (18/305)
20–29 (%)	9.5 (2/21)	11.1 (34/305)
30–39 (%)	38.1 (8/21)	23.0 (70/305)
40–49 (%)	9.5 (2/21)	20.3 (62/305)
50–59 (%)	38.1 (8/21)	25.9 (79/305)
60–69 (%)	4.8 (1/21)	17.0 (52/305)
≥ 70 (%)	0.0 (0/21)	3.6 (11/305)
Females (%)	47.6 (10/21)	58.7 (179/305)
Males (%)	52.4 (11/21)	41.3 (126/305)
Number of teeth	26.7 ± 2.7	26.3 ± 4.7
Number of caries	0.5 ± 1.2	0.6 ± 1.6
Number of fillings	13.8 ± 5.8	12.5 ± 5.7
Stimulated salivary flow (ml per 5 min)	7.3 ± 3.5	6.7 ± 6.3

Table 3. Oral malodour parameters in the *H. pylori*-positive and -negative subjects

Parameter		<i>H. pylori</i> -positive (n=21)	<i>H. pylori</i> -negative (n=305)	<i>P</i> value*
Oral malodour level				
H ₂ S	≥0.15 p.p.m.	90.5 (19/21)	75.1 (229/305)	NS
	<0.15 p.p.m.	9.5 (2/21)	24.9 (76/305)	
CH ₃ SH	≥0.50 p.p.m.	100 (21/21)	82.6 (252/305)	<0.05
	<0.50 p.p.m.	0 (0/21)	17.4 (53/305)	
(CH ₃)S	≥0.02 p.p.m.	81 (17/21)	72.8 (222/305)	NS
	<0.02 p.p.m.	19 (4/21)	27.2 (83/305)	
Total VSCs	≥0.25 p.p.m.	90.5 (19/21)	76.1 (232/305)	NS
	<0.25 p.p.m.	9.5 (2/21)	23.9 (73/305)	
OLT score	≥3	57.1 (12/21)	43.3 (132/305)	NS
	<3	42.9 (9/21)	56.7 (173/305)	
Clinical parameter				
Tongue-coating score	≥3	4.8 (1/21)	18.7 (57/305)	NS
	<3	95.2 (20/21)	81.3 (248/305)	
Caries	Presence	19.0 (4/21)	22.0 (67/305)	NS
	Absence	81.0 (17/21)	78.0 (238/305)	
No-good margin	Presence	14.3 (3/21)	7.5 (23/305)	NS
	Absence	85.7 (18/21)	92.5 (282/305)	
Tooth mobility	Presence	38.1 (8/21)	18.0 (55/305)	<0.05
	Absence	61.9 (13/21)	82.0 (250/305)	
PPD	≥5 mm	76.2 (16/21)	37.0 (113/305)	<0.01
	<5 mm	23.8 (5/21)	63.0 (192/305)	
	≥6 mm	57.1 (12/21)	29.8 (91/305)	<0.01
	<6 mm	42.9 (9/21)	70.2 (214/305)	
Occult blood in the saliva	Presence	66.7 (14/21)	36.7 (112/305)	<0.01
	Absence	33.3 (7/21)	63.3 (193/305)	
Periodontal bacteria				
<i>Porphyromonas gingivalis</i>	Presence	76.2 (16/21)	36.7 (112/305)	<0.01
	Absence	23.8 (5/21)	63.3 (193/305)	
<i>Treponema denticola</i>	Presence	85.7 (18/21)	54.4 (166/305)	<0.01
	Absence	14.3 (3/21)	45.6 (139/305)	
<i>Prevotella intermedia</i>	Presence	66.7 (14/21)	26.2 (80/305)	<0.01
	Absence	33.3 (7/21)	73.8 (225/305)	

* χ^2 test. NS, Not significant.

in the saliva were significantly greater in the *H. pylori*-positive subjects. The prevalence of *Porphyromonas gingivalis*, *Treponema denticola* and *Prevotella intermedia* in the *H. pylori*-positive subjects was 76.2, 85.7 and 66.7%, respectively. These periodontopathic bacteria were detected more frequently with *H. pylori* ($P < 0.01$). Ishihara *et al.* (1997) reported that *Porphyromonas gingivalis* and *F. nucleatum* selectively co-aggregate with *H. pylori*. Various oral bacterial species, however, are able to inhibit the growth of *H. pylori* (Okuda *et al.*, 2003). The fact that subjects with good oral hygiene harbour less *H. pylori* in their mouths can be explained by the inhibitory activity of early colonizers of dental biofilm, such as oral streptococci, against that species. Periodontopathic bacteria, including *Porphyromonas gingivalis*, *Treponema denticola* and *Prevotella intermedia*, produce H_2S from L-cysteine and CH_3SH from L-methionine (Fukamachi *et al.*, 2005; Tonzetich & McBride, 1981). *H. pylori* is capable of

producing H_2S and CH_3SH , although the production of these compounds is complicated and strain specific (Lee *et al.*, 2006).

Prevalence of oral *H. pylori* and oral malodour in periodontal subjects

Subjects with a tongue-coating score of ≥ 3 had more actual malodour (≥ 0.25 p.p.m. total VSCs) than the subjects with a tongue-coating score of ≤ 2 ($P < 0.01$), but no correlation was observed between carriage of *H. pylori* and tongue-coating score. To evaluate the relationship between *H. pylori* and oral malodour according to periodontal status, those subjects with a PPD of ≥ 5 mm and a tongue coating score of ≤ 2 ($n=102$) were selected (Table 4). Both the concentration of VSCs and the OLT scores were higher in the *H. pylori*-positive subjects, but the differences between the *H. pylori*-positive and -negative

Table 4. Oral malodour parameters in the *H. pylori*-positive and -negative subjects with a PPD of ≥ 5 mm and a tongue coating score of ≤ 2

Parameter		<i>H. pylori</i> -positive ($n=16$)	<i>H. pylori</i> -negative ($n=86$)	<i>P</i> value
Age (years)		45.7 $\pm$ 11.7	52.4 $\pm$ 11.7	<0.05*
Number of teeth		26.4 $\pm$ 2.6	25.8 $\pm$ 4.7	NS
Stimulated salivary flow (ml per 5 min)		7.2 $\pm$ 3.6	6.8 $\pm$ 3.2	NS
Oral malodour levels				
H_2S	≥ 0.15 p.p.m.	100.0 (16/16)	82.6 (71/86)	NS
	<0.15 p.p.m.	0 (0/16)	17.4 (15/86)	
CH_3SH	≥ 0.50 p.p.m.	100.0 (16/16)	93.0 (80/86)	NS
	<0.05 p.p.m.	0 (0/16)	7.0 (6/86)	
CH_3SCH_3	≥ 0.02 p.p.m.	93.8 (15/16)	90.7 (78/86)	NS
	<0.02 p.p.m.	6.25 (1/16)	9.3 (8/86)	
Total VSCs	≥ 0.25 p.p.m.	93.8 (15/16)	88.4 (76/86)	NS
	<0.25 p.p.m.	6.25 (1/16)	11.6 (10/86)	
OLT score	≥ 3	68.8 (11/16)	61.6 (53/86)	NS
	<3	31.2 (5/16)	38.4 (33/86)	
Clinical parameters				
Caries	Presence	18.8 (3/16)	29.1 (25/86)	NS
	Absence	81.2 (13/16)	70.9 (61/86)	NS
No-good margin	Presence	12.5 (2/16)	12.8 (11/86)	NS
	Absence	87.5 (14/16)	87.2 (75/86)	
Tooth mobility	Presence	43.8 (7/16)	36.0 (31/86)	NS
	Absence	56.2 (9/16)	64.0 (55/86)	
Occult blood in the saliva	Presence	87.5 (14/16)	50.0 (43/86)	<0.01†
	Absence	12.5 (2/16)	50.0 (43/86)	
Periodontal bacteria				
<i>Porphyromonas gingivalis</i>	Presence	81.2 (13/16)	67.4 (58/86)	NS
	Absence	18.8 (3/16)	32.6 (28/86)	
<i>Treponema denticola</i>	Presence	87.5 (14/16)	69.8 (60/86)	NS
	Absence	12.5 (2/16)	30.2 (26/86)	
<i>Prevotella intermedia</i>	Presence	81.2 (13/16)	47.7 (41/86)	<0.05‡
	Absence	18.8 (3/16)	52.3 (45/86)	

NS, Not significant.

*Significant difference between the *H. pylori*-positive and -negative subjects at $P < 0.05$ (unpaired *t*-test).

†Significant difference between the *H. pylori*-positive and -negative subjects at $P < 0.01$ (χ^2 test).

‡Significant difference between the *H. pylori*-positive and -negative subjects at $P < 0.05$ (χ^2 test).

subjects were not significant. In the patients with periodontitis, elevated numbers of other VSC-producing bacteria were present in the oral cavity (Tanaka *et al.*, 2004). Further detailed analysis should be performed to explore the relationship between the number of *H. pylori* present and halitosis levels. Note that the amount of occult blood in the saliva was greater in the *H. pylori*-positive subjects ($P < 0.01$). *H. pylori* has been suggested to have a special preference for the activated state of inflammation in periodontitis and several studies have reported that carriage of *H. pylori* may be associated with periodontal disease (Gebara *et al.*, 2006; Okuda *et al.*, 2003; Souto & Colombo, 2008). Our results suggest that the presence of *H. pylori* in the oral cavity may be related to halitosis through periodontal pocketing and inflammation, rather than VSC-producing ability. The prevalence of *Prevotella intermedia* was higher in the *H. pylori*-positive subjects ($P < 0.05$), whilst the prevalence of *Porphyromonas gingivalis* and *Treponema denticola* was independent of the presence of *H. pylori* in those subjects with periodontal symptoms. *Prevotella intermedia* has been detected in various parts of the oral cavity, including periodontal pockets, tongue debris and supragingival plaque. *Prevotella intermedia* ATCC 25611, however, failed to co-aggregate with *H. pylori* *in vitro* (Ishihara *et al.*, 1997), although the human oral cavity contains more than 500 bacterial species that interact with each other and with the tissues of their host (Kroes *et al.*, 1999; Paster *et al.*, 2001). The composition of these complex microbial communities varies in different ecological niches, such as the gingival crevice, tongue, buccal mucosa and saliva. Additional research is needed to confirm the local existence of these organisms.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid for Scientific Research (C) 18592296 (to T.H.), 20592249 (to M.Y.) and 20592444 (to T.N.); a Grant-in-Aid for Young Scientists (B) 19791645 (to N.S.); and Grants-in-Aid for Frontier Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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