**The role of Solobacterium moorei in oral malodour**

For figures, tables and references we refer the reader to the original paper.

Introduction

Halitosis has most often (80–90%) an intra-oral origin (Quirynen et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26), Vandekerckhove et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib45)), resulting from an intra-oral microbial degradation of organic substrates by anaerobic bacteria. It is believed that the dorso-posterior region of the tongue is the primary source of oral malodour (Tonzetich [1977](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib42), Loesche and Kazor [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib20)). Oral malodour is indeed, in the majority of the cases, caused by tongue coating (Quirynen et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26)). Anaerobic bacteria in the tongue coating produce volatile compounds, of which the sulfur compounds (H2S, CH3SH and (CH3)2S) are the most extensively studied (Tonzetich [1977](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib42), Delanghe et al [1997](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib5)). Treponema denticola, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, Bacteroides loescheii, Enterobacteriaceae, Tannerella forsythensis, Centipeda periodontii, Eikenella coorodens, Fusobacterium nucleatum are identified as key-bacteria in the above mentioned process (Awano et al [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib1)).

In 1966, Gordon and Gibbons were the first to explore the tongue microbiota (Gordon and Gibbons [1966](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib11)). Since then, most of the studies concluded that the tongue microbiota is characterized by a wide variety of bacteria, with high proportions of the above mentioned volatile sulfur compounds (VSC) producing anaerobic species (Loesche and Kazor [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib20)). Unfortunately, the bacterial composition of the tongue dorsum is still not fully determined. The introduction of molecular approaches has expanded our insight in the tongue microbiota with an increase in complexity (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16)). Recently Solobacterium moorei has received increased interest.

S. moorei, a non-spore-forming Gram-positive anaerobic bacillus, was originally isolated from human faeces. In the past, S. moorei was wrongly classified as a Eubacterium-strain, because of difficulties in phenotypical characterization. DNA sequencing seems necessary for the identification of S. moorei in clinical samples (Zheng et al [2010](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib49)), more specifically 16S rRNA gene sequencing (Kageyama and Benno [2000](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib15)). S. moorei is the only species in the genus Solobacterium belonging to the Clostridium cluster XVI. As such it has also been associated with bacteraemia (Lau et al [2006](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib18)), necrobacillosis-associated thrombophlebitis (Martin et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib22)), septicemia (Detry et al [2006](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib6)), and wound infection (Zheng et al [2010](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib49)). In dentistry S. moorei can be found in endodontic infections (Downes et al [2001](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib9), Rolph et al [2001](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib30), Rôças et al [2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib28)), periradicular lesions (Schirrmeister et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib34)), and subgingival plaque of patients with refractory periodontitis (Colombo et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib3)). Recently, this bacterial species was associated with oral malodour (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16), Haraszthy et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib14), [2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib13)).

Considering the importance of the oral cavity as a reservoir for micro-organism causing oral malodour (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16), Roldán et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib29), Tanaka et al [2004](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib38)) and the association proposed between S. moorei and oral malodour, the aim of this study was to analyse the correlation between S. moorei and a wide variety of clinical and halitosis parameters.

Materials and methods

Subjects

Data from 193 patients, who visited our specialized halitosis clinic (University Hospital Leuven, department periodontology, Belgium) were considered for this study. Permission was obtained from the local Ethical Committee (s54747). The data were collected from a standard protocol. All subjects had bad breath complaints. Subjects who had used antibiotics two months prior to the consultation and extra-oral causes of halitosis were excluded. Prior to the consultation, all subjects were provided with following instructions: two days before their appointment the patients were asked to refrain from eating garlic, onions or spicy food, to avoid alcohol or coffee and smoking 12 h prior to the examination, and not to use chewing gum, mints, drops or mouth rinses on the day of the examination. On the other hand, in order to stand firm normal daily activity, oral hygiene and breakfast were allowed. All measurements were recorded in the morning between 08.15 and 11.30 am. Patients were informed about the research and gave their written consent for the analysis of the data.

Questionnaire

Before clinical examination, the patients were asked to complete a questionnaire regarding their general health, allergies, medication, smoking habits and oral hygiene. A complete medical history was recorded for each subject, paying special attention to systemic diseases that may compromise the lungs, liver, kidneys, stomach and/or pancreas. Information on inter dental cleaning, tongue cleaning, use of mouth rinses, presence of removable appliances etc were also retrieved via the questionnaire.

Clinical parameters

Organoleptic assessment

The intensity of oral malodour was determined by a trained and calibrated judge. She was previously tested on the ability to distinguish odours using the Smell Identification Test® (Sensonics Inc., Haddon Heights, NJ, USA) (Double et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib8)) and on the ability to detect odours at low concentrations using a series of dilutions of isovaleric acid (Doty et al [1984](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib7)). The breath was scored while patients were counting from 1 to 11 as described by Rosenberg ([1996](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib31)). The organoleptic score (OLS) ranged from 0 to 5, where 0 represents absence of odour, 1 barely noticeable odour, 2 slight malodour, 3 moderate malodour, 4 strong malodour and 5 severe malodour (Rosenberg and McCulloch [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib33)). To avoid any bias, organoleptic assessment preceded all other breath examinations.

VSC assessment

Mouth air was further analysed via a portable gas chromatograph (OralChroma®, Abilit Corporation, Kanagawa, Japan) to measure individual concentrations in ppbv (nmol/mol) of three VSCs (H2S, CH3SH, (CH3)2S). After inserting 2/3 of a plastic disposable syringe (1 ml) in the oral cavity, subjects were asked to close their mouth for 30 s. A volume of 0.5 ml mouth air was sampled and injected into the device. After 8 min, the concentration of the three gases was displayed. Because of the limitations of the device, disclosed by Tangerman and Winkel ([2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib40)), each chromatogram (retention time and peak shape) was visually reviewed before the results were accepted.

Mouth air was also examined with a portable sulfur monitor (Halimeter®, Interscan, Chatsworth, USA), measuring the total concentration of VSC's (Rosenberg et al [1991](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib32)). This electronic device aspirates the mouth air through a disposable straw, connected to the inlet of the device. After the patients kept their mouth closed for 60 s, the straw was inserted in the oral cavity. The examiner kept the straw from touching the tongue or other oral surfaces while the sample was collected. During sampling, patients were asked to hold their breath. Peak values were calculated by the computer and also visually reviewed. Measurements were done in duplicate. As in previous studies of our group, peak values were used for the calculations.

Intra-oral examination

Oral hygiene was scored based on the Silness and Loë plaque index, giving a three-point scale with 0 for perfect hygiene, 1 for moderate, and 2 for bad hygiene (Silness and Loë [1964](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib35)). The periodontal status small was scored via the Dutch Periodontal Screening Index (Van der Velden [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib43)). The latter was used to classify the patients as periodontally healthy (score 0), gingivitis (score 1 or 2), and periodontitis (score 3± or 4). The tonsils were examined for the presence of swelling, crypts, and/or tonsilloliths.

The degree of tongue coating was assessed on several ways. First of all three indices were applied: the Miyazaki index (MTCI) (Miyazaki et al [1995](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib23)), the Winkel index (WTCI) (Winkel et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib46)) and a modification of the Winkel index (mWTCI) (Lundgren et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib21)). Miyazaki proposed to assess the tongue coating according to the area involved: 0 for no coating, 1 if less than 1/3, 2 if less than 2/3, and score 3 when more than 2/3 of the tongue dorsum is covered (Miyazaki et al [1995](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib23)). More recently, Winkel and co-workers (Winkel et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib46)) described a new index where besides the surface, also the thickness of the tongue coating is evaluated. They divide the surface of the tongue in six areas, i.e. three in the posterior and three in the anterior part. The tongue coating in each sextant is scored as 0 being no coating, 1 for a light-thin coating, and 2 in case of a heavy-thick coating. Lundgren and co-workers (Lundgren et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib21)) discovered that Winkel score 1 often represents an increased keratinization of the papillae rather than tongue coating. Since this may affect the validity of the index, they eliminated score 1 in their approach.

To weight the tongue coating, all coating was removed with a plastic commercial scraper (Halita®, Dentaid, Spain) and collected into a plastic container (Yaegaki and Sanada [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib48)). To avoid saliva, cotton rolls were placed under the tongue and the tongue dorsum was dried with gauze packs, and air spray before scraping. For the last 100 subjects, also the dry weight of the tongue coating was considered. After measuring the wet weight, the samples were kept open under a hood for a week and reweighted. All measurements were performed with a precision balance (Voyager®, Ohaus Corporation, Parsippany, USA) to nearest 0.1 mg.

The flow rate of stimulated and non-stimulated saliva was determined in ml per minute, and its pH was recorded with a pH meter (WTW® ProfiLine pH 3110, Germany). For the non-stimulated saliva, patients were asked to spit in a container, every 30 s during 5 min, while resting. For the stimulated flow rate, patients were instructed to do the same but now while chewing on a standardized piece of paraffin, trying to produce as much saliva as possible (Laine et al [1999](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib17)).

Microbiological sampling and DNA probe assay

In all patients, microbiological samples were collected from both the saliva and the tongue dorsum. Before determining the saliva flow, patients were asked to spit twice in a container. From this container 0.2 ml was taken and diluted in 2 ml reduced transport fluid (RTF). Samples from the tongue dorsum were collected, after drying the tongue (see above), by gently moving a cotton swab over an area of ±2 cm2 of the posterior part. The cotton swab was also preserved in RTF during transportation to the lab. S. moorei was identified by quantitative polymerase chain reaction (qPCR) as follows: DNA was extracted from bacterial samples with DNeasy Tissue Kit (QIAGEN Ltd., Venlo, The Netherlands) in accordance with the manufacturer's instructions. A qPCR assay was performed with a CFX96 Real-Time System (Biorad, Hercules, CA, USA). The Taqman 5' nuclease assay PCR method was used for detection and quantification of bacterial DNA. Primers and probes were targeted against the 16S rRNA gene. Taqman reactions contained 12.5 µl Mastermix (Eurogentec, Seraing, Belgium), 4.5 µl sterile H2O, 1 µl forward primer (900 nM), reverse primer (900 nM) and probe (200 nM) and 5 µl template DNA. Assay conditions consisted of an initial 2 min at 50 °C, followed by a denaturation step for 10 min at 95 °C, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Quantification was based on a plasmid standard curve. For this a fragment of the 16S rRNA gene of S. moorei JCM 10645 was amplified with primers flanking the annealing site of the qPCR primers (table [1](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t1)). The fragments were ligated into the pGEM-T easy vector system (Promega, Madison, WI, USA) and used to transform Escherichia coli DH5α. Plasmids were isolated from the clones with the High Pure Plasmid Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany). The concentration of the plasmid was determined using a GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech, Roosendaal, the Netherlands) at a wavelength of 260 nm.

A ten-fold dilution series of the plasmid was used in each qPCR run to construct the standard curve. Data were collected during each annealing phase. In each run, no template controls were included. Results were expressed as log10 genome equivalents gEq ml–1 or as number of bacterial genomes ml–1. All microbiological evaluations were performed blind.

Statistical analysis

In order to establish the relation between S. moorei and the different parameters, Spearman correlations and their corresponding p-values were determined. We considered p-values of <0.05 to be significant. A multiple regression model selection was constructed of all parameters, thought to be statistically and clinically relevant, including the different tongue coating indices (WTCI, mWTCI, MTCI, wet/dry weight), periodontal indices (oral hygiene, gingivitis, periodontitis), saliva parameters (pH, flow rate) and S. moorei (tongue and saliva).

Results

Patient sample

Data from 193 consecutive patients, visiting a multidisciplinary halitosis clinic (University Hospital Leuven, department periodontology, Belgium) were included in the analysis. There were slightly more females (56%) than males. The subject's age ranged from 15 to 76 years (mean: 40.5 yr). Only small portions were smokers (12%) and almost half of the subjects (42%) presented with good oral hygiene. Even though subjects visited a specialty halitosis consultation, the proportion of OLS ≥ 3 was relatively small (43/193, or 22.3%), whereas 27.5% of the subjects (53/193) presented with an OLS of 0. Tonsils were removed in 30% of the subjects; most subjects with tonsils did not show abnormalities in tonsil anatomy. In 3% of the cases, tonsilloliths could be detected.

For the analysis, subjects were divided over two subgroups (table [2](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t2)): those with an OLS ≤ 1 (none or barely noticeable odour, called the 'no-malodour group', n = 109), and those with an OLS ≥ 2, the 'malodour group', n = 84 (Murata et al [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib24)).

The descriptive statistics of the two subgroups are summarized in table [3](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t3). The questionnaire did not really highlight major differences between both subgroups, with the exception of a slightly larger proportion of smokers, tongue cleaners, and users of a mouthrinse in the no-malodour group. However, when oral hygiene and periodontal health were objectively recorded, it became obvious that the no-malodour group had a larger proportion of patients with a good oral hygiene (59.6% versus 20.2%), and with periodontal health (66.9% versus 19.0%).

Table 3. Descriptive statistics of study population, grouped for patients with and without

Clinical parameters

The different breath and saliva parameters, according to the two subgroups, are displayed in table [4](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t4). The concentration of H2S and CH3SH was ten times higher in the malodour group, compared to the no-malodour group, the observed amounts and differences for (CH2)3S were rather small. Also the total amount of VSCs measured by the Halimeter® was three times higher in the oral malodour group. The different tongue coating indices indicated clearly higher scores for the malodour group. The latter group also showed a nearly double wet and dry tongue coating weight (209.7 versus 132.7 mg, 8.7 versus 5.3 mg, respectively). Neither the salivary flow rate, nor its pH showed large differences between both subgroups. The incidence of S. moorei positive patients is higher in the malodour group (98.8% versus 88.9% for tongue and 97.6% versus 90.8% for saliva, respectively), as well as the overall mean numbers (4.06 versus 3.34 for tongue and 4.0 versus 3.22 for saliva, respectively).

Table 4. Frequency distribution of different parameters (VSC levels, tongue coating, saliva) and qPCR data, per subgroup.

Spearman correlation coefficients between breath parameters and potential aetiological factors

Table [5](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t5) summarizes the correlation between several breath parameters (OLS, H2S and CH3SH) and potential aetiological parameters for oral malodour. OLS and H2S correlate significantly with many and always the same parameters (tongue coating indices, periodontal parameters and the concentration of S. moorei), except for saliva parameters, whereas CH3SH correlated with only a few.

Table 5. Spearman correlations between OLS, H2S and CH3SH and potential aetiological factors (tongue coating indices, periodontal indices, saliva parameters and concentration of S. moorei in saliva and on the tongue). Significant p values are in bold; r represents Spearman rank correlation coefficients.

The strongest Spearman rank correlation coefficients were found between H2S and S. moorei concentration on the tongue (r = 0.59), OLS and oral hygiene (r = 0.55), OLS and S. moorei concentration in the saliva (r = 0.51) and OLS and S. moorei concentration on the tongue (r = 0.51). CH3SH on the other hand only correlated with gingivitis and the concentration of S. moorei. Some significant negative correlations could be found between periodontal health, saliva parameters (flow rate and pH) on one hand, and OLS, H2S, CH3SH on the other hand. An additional significant spearman rank coefficient was calculated between dry and wet weight of tongue coating (r = 0.81, p <0.0001).

Spearman correlation coefficients between S. moorei and breath parameters or potential aetiological factors

Correlations between S. moorei and OLS, VSC's, tongue coating indices, periodontal indices, oral hygiene, smoking and S. moorei are displayed in table [6](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t6). A strong significant correlation could be found between S. moorei and OLS, H2S, CH3SH (see above), the correlations were weaker, although still significant for (CH3)2S, total VSC, oral hygiene, tongue coating indices and gingivitis/periodontitis. The clearest significant correlation was found between S. moorei concentration on the tongue and H2S (r = 0.59), followed by the total VSC measured with the Halimeter and S. moorei in saliva (r = 0.58). Tongue coating indices (MTCI, WTCI, mWTCI) correlated with both S. moorei in saliva as of the tongue, but best correlations are seen with S. moorei of the tongue. Dry weight of the tongue coating correlated significantly with S. moorei on the tongue and saliva, 0.37 and 0.27 respectively, only S. moorei concentration on the tongue has a correlation with wet weight. Both gingivitis and periodontitis correlated significantly with S. moorei. A negative correlation was found between S. moorei and smoking. The presence of a removable prosthesis could not be correlated with S. moorei.

Table 6. Spearman correlations coefficient between S. moorei and breath parameters and potential aetiological factors.

Multiple regression analyses

The results of the multiple regression analyses are summarized in table [7](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t7). This analysis of OLS, H2S and CH2SH and clinical variables (see above) showed that a combination of the concentration of S. moorei, especially on the tongue, oral hygiene and tongue coating (WTCI) explained most of the variation in OLS, H2S and CH3SH. S. moorei explains a significant part of the variability of the parameters OLS, H2S and CH3SH, for 14.0%, 21.6% and 15.3% respectively.

Table 7. Multiple regression analysis to explain the variability in OLS, H2S and CH3SH, respectively. The log10 value of the concentration of S. moorei on the tongue was used.

Discussion

This clinical study contained data from 193 consecutive patients, visiting a bad breath consultation. Based on the OLS, patients were divided in two subgroups (OLS ≤ 1 (n = 109) and OLS ≥ 2 (n = 84)) (Murata et al [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib24)). The subgroup defined as no-malodour is larger than the malodour group (table [2](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t2)). This might be explained by the important group of patients with pseudo-halitosis and halitophobia. The term pseudo-halitosis is used when, even though no objective diagnosis of halitosis could be perceived, the patient is convinced that he/she suffers from bad breath. If after a diagnosis of pseudo-halitosis, the patient is still convinced they have bad breath, the term halitophobia applies (Yaegaki and Coil [2000](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib47)). A study of 2000 patients in Leuven showed that pseudo-halitosis or halitophobia could be diagnosed in 16% of the cases, were in a first report in 1997 (Delanghe et al [1997](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib5)), this group of patients was not even reported. More than 2/3 of these patients were women (Quirynen et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26)). According to our data there were more women in the no-malodour group (table [3](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t3)). The fact that the total amount of our 193 patients with OLS ≥ 3 was rather small (22.3%), was also similar to the study of Quirynen et al ([2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26)), where only 19.9% of the patients had OLS ≥ 3.

The mean values of H2S and CH3SH was clearly higher in the malodour group (OLS ≥ 2), compared with the no-malodour group (OLS ≤ 1), which was not the case for (CH3)2S (table [4](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t4)). This is explained by the fact that (CH3)S2 appears to correlate better with extra-oral halitosis (Tangerman and Winkel [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib39)). During the study period, no extra-oral causes of halitosis were included.

The reason why tongue coating was assessed, is because the dorso-posterior region of the tongue seems to be the primary source of malodour (Tonzetich [1977](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib42), Yaegaki and Sanada [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib48)). Quirynen and co-workers found tongue coating as the major aetiological factor for oral malodour (43%) (Quirynen et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26)). Other studies found a significant correlation between oral malodour and tongue coating (Rosenberg [1996](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib31), Liu [2006](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib19), Vandekerckhove et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib45)). In a more recent study of 96 patients, tongue coating seemed to correlate with several halitosis parameters, especially oral hygiene (Van Tornout [2013](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib44)). These findings are in agreement with this study, since the high values of all three tongue coating indices (Miyazaki > 2/3, Winkel between 10–12 and Modified Winkel ≥4), are clearly more frequently observed in the oral malodour group compared to the no-malodour group (table [3](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t3)). Also the weight of tongue coating differed between both groups. The mean value of both wet and dry weight was higher in malodour group. The dry weight was included in order to see if there would be a difference between the wet and dry weight of tongue coating. Out of the significant correlation between those two parameters (r = 0.81), we can assume that the difference is small.

Previous studies indicated the colonization of the dorso-posterior surface of the tongue by S. moorei in halitosis patients (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16), Haraszthy et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib14)). In accordance with these studies, a higher amount and detection frequency of S. moorei was found in patients with oral malodour. This correlation can be explained by the fact that S. moorei produces VSCs, more specific H2S (Haraszthy et al [2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib13)), but also produces β-galactosidase. β-galactosidase is an enzyme that is responsible for the deglycosylation of salivary glycoproteins, a process that can result in an increased oral malodour (Sterer and Rosenberg [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib37)). A significant correlation between OLS/tongue malodour and β-galactosidase has been demonstrated (Sterer [2002](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib36)). There are more bacteria associated with oral malodour, also able to produce β-galactosidase, including P. gingivalis, P. intermedia and P. nigrescens (Fournier and Mouton [1993](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib10), Haraldsson and Holbrook [1999](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib12)).

Although the role of CH3SH in patients with periodontitis has been shown in other studies (Yaegaki and Sanada [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib48), Coli and Tonzetich [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib2)), no strong correlation between CH3SH and periodontitis was found (table [5](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t5)). The severity of periodontitis seems to be important since the VSC concentration increases with the total pocket depth (Tonzetich [1973](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib41)). Moreover, Yaegaki and Sanada showed that the CH3SH/H2S ratio increased with the probing depth (Yaegaki and Sanada [1992](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib48)). The reason for not finding this correlation might be the rather small amount of subjects in our study with periodontitis (table [3](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t3)).

Significant correlations could be established between OLS/H2S, tongue coating and periodontal indices. This reinforces the role of tongue coating and periodontal disease in oral malodour (Miyazaki et al [1995](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib23), De Boever et al [1995](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib4), Quirynen et al [1998](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib27), [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib26), Oho et al [2001](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib25), Vandekerckhove et al [2009](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib45), Van Tornout [2013](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib44)).

Table [6](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t6) shows significant correlations between S. moorei and OLS, H2S, CH3SH, (CH3)2S and total VSC. In contrast to previous studies (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16), Haraszthy et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib14), [2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib13)), different parameters were included, a larger study population was examined, and not only the detection but also the concentration of S. moorei was recorded. Haraszthy and co-workers found a correlation between S. moorei and OLSs of r = 0.79 and for VSC levels of r = 0.69. The latter may be explained by the selection criteria for the malodour group with a higher proportion of severe (OLS ≥ 3) subjects (Haraszthy et al [2008](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib13)). Next to breath parameters, S. moorei showed also a significant correlation with tongue coating indices (table [6](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t6)). Kazor and co-workers determined the bacterial diversity on the tongue dorsum using culture-independent molecular methods and found S. moorei in 3/6 subjects with halitosis (OLS ≥ 2) and only 1/5 control subjects (Kazor et al [2003](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib16)). In a comparable study, Haraszthy and co-workers observed that S. moorei was the only bacterial species present in all of the eight subjects with halitosis (OLS ≥ 3), in proportions as high as 10.8%, but in none of the five control subjects, suggesting that some subjects with halitosis harbour some distinct bacterial species on their tongue dorsum (Haraszthy et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib14)). Their most recent study confirmed this idea, by detecting S. moorei in all of the 21 subjects with halitosis (OLS ≥ 3), and in only 5/36 control subjects. S. moorei was the seventh most numerous (4.8%) of the 2768 bacterial species from halitosis tongue dorsum samples (Haraszthy et al [2007](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357bib14)). In contrast to previous studies, we could also detect S. moorei, both from the tongue as in saliva (88.9% and 90.8% respectively) in the no-malodour group (table [4](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t4)). A plausible explanation could be the larger population and the less strict oral malodour criteria (OLS ≥ 2) established in our study.

Out of the questionnaire, a well-defined negative correlation between smoking behaviour and S. moorei was registered. However, the low number of smokers in our population (12%) must be kept in mind.

From the results of the multiple regression analysis, we could conclude that a relation exists between OLS and tongue coating (Winkel index), oral hygiene and S. moorei of the tongue (table [7](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t7)). This is confirmed by the Spearman correlations (table [5](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t5)) (r = 0.43; r = 0.55 and r = 0.51 respectively). Those three parameters are also found for H2S and CH3SH (table [7](http://iopscience.iop.org/1752-7163/7/4/046006/article#jbr473357t7)).

The fact that S. moorei is only slightly increased in the oral malodour group, could raise the question whether there is a link with other VSC producing bacteria. Could it be that S. moorei increases when other bacterial species are present? Further research examining the correlation between S. moorei and other VSC producing bacteria could be interesting to clarify the role of these specific bacteria.

Conclusion

In this study, a clear correlation between OLS/H2S/CH3SH, tongue coating and periodontal indices was found. We could also establish a significant correlation between OLS, VSCs, tongue coating-, periodontal indices and S. moorei, from which we can suggests that its presence of in the oral cavity might be associated with oral malodour.

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The authors declare no conflict of interest