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Short communication

Characterization of volatile sulfur compound production by *Solobacterium moorei*

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ARTICLE INFO

Article history:
Accepted 20 September 2012

Keywords:
Halitosis
Volatile sulfur compounds
Mucin
Trypsin
Solobacterium moorei

ABSTRACT

Objective: *Solobacterium moorei* is a Gram positive bacterium that has been specifically associated with halitosis. The aim of this study was to characterize volatile sulfur compound (VSC) production by *S. moorei*.

Methods: *S. moorei* was either grown or incubated in the presence of various supplements prior to determining VSC production with a Halimeter sulfide monitor. The effect of exogenous proteases or glycosidase inhibitors on VSC production by *S. moorei* was examined.

Results: We first showed that *S. moorei* can convert cysteine into hydrogen sulfide. The capacity of *S. moorei* to produce VSCs from serum, saliva, and mucin was dependent on the presence of an exogenous source of proteases such as pancreatic trypsin or *Porphyromonas gingivalis* gingipains. VSC production from mucin was inhibited by the presence of a β-galactosidase inhibitor, thus suggesting that deglycosylation of mucin by *S. moorei* is critical for VSC production.

Conclusion: Our study suggests that *S. moorei* can be a major source of malodorous compounds in halitosis by producing VSCs through a process involving the β-galactosidase activity of the bacterium and an exogenous source of proteases.

1. Introduction

Halitosis is a relatively common condition in which a person either suffers from bad breath or perceives himself/herself as having offensive mouth odour (halitophobia). Halitosis can have multiple causes, but in most cases, bad breath originates from the oral cavity.[1,2] Extraoral causes of bad breath are less common, and include nasal and pharyngeal infections, respiratory conditions, gastro-intestinal problems, metabolic conditions like diabetes, and liver diseases.[3] Bad breath originating from the oral cavity is due to by-products generated through bacterial metabolic degradation. For individuals with a healthy oral cavity, an important site for accumulation of bacteria associated with halitosis is the dorsum of the tongue and specially its posterior portion, which provides a suitable environment for growth of anaerobes since a low oxygen concentration is present in the deep crypts of the tongue.[3] Poor oral hygiene that results in accumulation of bacterial biofilm in subgingival areas and that may lead to periodontal disease has also been associated with halitosis.[4]

Volatile sulfur compounds (VSCs), which include hydrogen sulfide, methyl mercaptan and dimethyl sulfide, have been

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0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.09.011

regarded as the primary compounds responsible for halitosis originating from the oral cavity, although amines (cadaverine, putrescine and indole) and short-chain fatty acids (isobutyric and isovaleric acids) also contribute to oral malodor.[5] VSCs are generated through an enzymatic modification of sulfur-containing amino acids (cysteine and methionine), which are made available following proteolytic degradation of proteins or glycoproteins.[5] Bacteria that have been classically associated with halitosis include *Fusobacterium nucleatum*, *Prevotella intermedia*, *Veillonella alcalescens*, *Porphyromonas gingivalis* and *Treponema denticola*.[5] The large array of hydrolytic enzymes produced by these bacteria are likely to act in synergy to produce VSCs.[6] Recently, the Gram positive anaerobic bacterium *Solobacterium moorei* (formerly known as *Bulleidia moorei*) has been specifically associated with oral malodor since it has been reported to be present in subjects with halitosis but not in control subjects.[7–10] More particularly, Haraszthy et al.[7] detected *S. moorei* in 100% of the 21 subjects with halisosis compared to only 14% of the control subjects. It is worth mentioning that this bacterial species has also been associated with wound infections and bacteremia.[11,12] To the best of our knowledge, the production of VSCs by *S. moorei* has not been documented. Therefore, the aim of this study was to characterize VSC production by *S. moorei*.

2. Materials and methods

2.1. Bacteria and culture conditions

S. moorei SUNYAB H8-20, kindly provided by Haraszthy et al. (The State University of New York at Buffalo), was used in this study. This strain was isolated from the dorsal surface of the tongue in a subject with halitosis.[7] Bacteria were routinely grown in Todd-Hewitt Broth (THB) medium (BBL Microbiology Systems, Cockeysville, MD, USA) containing 0.001% hemin, 0.0001% vitamin K, 0.5% Tween-80, 0.2% yeast extract, and 1% glucose. Incubation was carried out at 37 °C under anaerobic conditions (N_2:H_2:CO_2/80:10:10).

2.2. Effects of methionine, cysteine, serum, saliva, and mucin as growth supplement on VSC production

The effect of supplementing the above culture medium with methionine (5 and 25 mM), cysteine (5 and 25 mM), heat-inactivated (60 °C/30 min) foetal bovine serum (2 and 10%), heat-inactivated human saliva (2 and 10%) or mucin (0.5 and 2 mg/mL; porcine stomach type III) on VSC production by *S. moorei* was evaluated. VSC levels of the test tube headspace were determined, as described below, after 24 h of growth into sealed screw cap tubes. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Non-stimulated saliva collected from five healthy volunteers was pooled, filtrated and inactivated at 60 °C for 30 min.

2.3. Effect of exogenous proteases on VSC production

Cells of *S. moorei* were harvested from a 24-h culture and suspended in 50 mM phosphate-buffered saline pH 7.2 (PBS) to obtain an OD_{660} of 2.0. Equal volumes of the bacterial suspension and mucin ± pancreatic trypsin (Sigma Chemical Co.) were placed into sealed screw cap tubes. Final concentrations of mucin and trypsin were 0.5 and 2 mg/mL, and 0.05 and 0.25 mg/mL, respectively. Assay mixtures were incubated at 37 °C for 4 h, and VSC levels of the test tube headspace were determined as described below. The assay was also carried out in the presence of glucose (10%) and/or the β-galactosidase inhibitor *p*-aminophenyl-β-D-thiogalactopyranoside (5 and 10%). The effect of trypsin or a purified preparation of *Porphyromonas gingivalis* Arg-gingipains, isolated as described in a previous study,[13] on VSC production from heat-inactivated serum and saliva was also evaluated.

2.4. Determination of VSC levels

VSC levels were assessed using a Halimeter sulfide monitor (Interscan Corp., Chatsworth, CA, USA). This portable monitor has been reported to be more sensitive to hydrogen sulfide than to methyl mercaptan.[14] Briefly, the monitor was zeroed on ambient air and a 0.25 in. diameter disposable plastic straw was attached to the air inlet of the monitor. VCS levels of the test tube headspace were measured by inserting the other end of the straw 2 cm into each test tube immediately after removing the cap and recording the maximum reading in parts per billion (ppb) sulfide equivalents.

2.5. Determination of proteolytic activities

Cell-associated and extracellular proteolytic activities of *S. moorei* were determined using synthetic peptides labelled with *p*-nitroaniline (*p*Na) (Sigma Chemical Co.) and self-quenched gelatin labelled with fluorescein (DQ gelatin; Molecular Probes, Eugene, OR, USA). The chromogenic synthetic peptides used were N-α-benzoyl-DL-arginine-*p*Na (for trypsin-like activity), N-valyl-leucine-lysine-*p*Na (for plasmin-like activity), and N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-*p*Na (for chymotrypsin-like activity). Bacterial suspension (OD_{660} = 2.0 in PBS) or culture supernatant (100 μL) was incubated with 20 μl of each of the substrate (2 mg/mL). The reaction mixtures were incubated at 37 °C for 4 h prior to determine the absorbance at 405 nm (A_{405}) or the fluorescence at an excitation wavelength of 490 nm and emission wavelength of 520 nm with a microplate reader, after removing bacteria by centrifugation when appropriate.

2.6. Determination of β-galactosidase activity

β-Galactosidase activity of *S. moorei* (cells and culture supernatant) was determined as described above using the chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (Sigma Chemical Co.). Following incubation for 2 h at 37 °C the absorbance at 420 nm was recorded.

2.7. Statistical analysis

All assays were performed in triplicate and the means ± standard deviations were calculated. Differences between means were analysed for statistical significance using the Student's *t*-test and were considered significant at $P < 0.05$.