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The bacterial metabolic processes that produce volatile sulfur compounds in the oral cavity

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THE BACTERIAL METABOLIC PROCESSES THAT PRODUCE VOLATILE SULFUR COMPOUNDS IN THE ORAL CAVITY

by

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2014
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DEDICATION

I would like to dedicate this work to my parents Mazen and Iman.
ACKNOWLEDGMENTS

I would like to thank Drs. Franzblau and Helmerhorst for their help and support.
THE BACTERIAL METABOLIC PROCESSES THAT PRODUCE VOLATILE SULFUR COMPOUNDS IN THE ORAL CAVITY

OSAMA S., DUWAJI

ABSTRACT

Volatile sulfur compounds are the primary cause of bad breath. They are a byproduct of bacterial metabolism and can be difficult to eliminate because they generally originate on the dorsum of the tongue, an area often missed during oral hygiene practices.

Chronic bad breath, or halitosis, can be a cause of extreme anxiety. Indeed, halitosis has been proven to affect people across the globe. However, doctors and dentists are generally unaware of the causes of this disease.

While poor oral hygiene is the most obvious cause of halitosis, many sufferers in fact have scrupulous oral hygiene practices. Little is known about the development of the disease; researchers have instead focused on which mouth rinses are the most effective and which bacteria are the most likely culprits.
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LIST OF ABBREVIATIONS

CHX................................................................. Chlorhexidine
CPC............................................................... Cetylpyridinium chloride
VSC............................................................... Volatile sulfur compounds
INTRODUCTION

For most people, bad breath is an occasional nuisance, dispelled by several invigorating seconds of gum-chewing. But for others, bad breath happens often enough to be considered a kind of disability.

Between 10% and 30% of all Americans suffer from bad breath on a regular basis, and no amount of gum chewing or tooth brushing makes it go away. These people have “Halitosis,” a term coined in 1874 but not really used till the 1920s when the first modern mouthwashes were invented (“Halitosis - Definition” n.d.) (Van der Sleen et al., 2010). Today, halitosis is a global phenomenon (see Table 1), but scientific research has not quite elucidated the causes behind this disease and the best methods for its eradication.
<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence of Halitosis</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>30%</td>
<td>(Yaegaki &amp; Sanada, 1992)</td>
</tr>
<tr>
<td></td>
<td>43% in &gt; 60 yrs old</td>
<td>(Steenberghe et al, 1996)</td>
</tr>
<tr>
<td>Japan</td>
<td>24%</td>
<td>(Miyazaki et al., 1995)</td>
</tr>
<tr>
<td>France</td>
<td>50-60%</td>
<td>(Meningaud et al., 1999)</td>
</tr>
</tbody>
</table>
| Netherlands | 25% in > 60 yrs old  
|          | 10% in < 20 yrs old                     | (de Wit, 1966)                                |
|          |                                         | (de Wit, 1966)                                |
| China    | 27.5%                                   | (X. N. Liu et al., 2006)                      |
| Switzerland | 32%                                     | (Bornstein et al., 2009)                     |
| Turkey   | 28% in > 60 yrs old                     | (Avcu et al., 2005)                           |
| Kuwait   | 25%                                     | (Al-Ansari et al., 2006)                      |
| Brazil   | 15%                                     | (Nadanovsky et al., 2007)                     |
Halitosis is generally divided into intra-oral and extra-oral. (In other words, originating from inside the oral cavity or coming from elsewhere). Intra-oral cases constitute approximately 90% of all cases of halitosis (Quirynen et al., 2009). Extra-oral halitosis, which can usually be identified by a clinician measuring nose-breath compared to mouth-breath, is indicative of some kind of serious systemic disease. See Table 2 for a list of such diseases.

**Extra-Oral Halitosis**

Causes of extra-oral halitosis include pathologies of the lungs and gastrointestinal tract, organ failure, metabolic disorders, and Ear-Nose-and-Throat disorders.

**Table 2. Metabolic and Endocrinological diseases that cause recognizable breath odor.** Adapted from van Steenberge. (van Steenberge D., 2009, n.d.)

<table>
<thead>
<tr>
<th>Metabolic or Endocrinological problem</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes, Ketoacidosis</td>
<td>Fruity odor</td>
</tr>
<tr>
<td>Intestinal Obstruction</td>
<td>Fecal odor</td>
</tr>
<tr>
<td>Kidney-insufficiency</td>
<td>Ammonia or Fishy Odor</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>Mouse odor</td>
</tr>
<tr>
<td>Methionine adenosyl transferase deficiency</td>
<td>Cooked cabbage odor</td>
</tr>
<tr>
<td>Maple Syrup Urine Disease</td>
<td>Burned Sugar odor</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>Sweet musty odor</td>
</tr>
<tr>
<td>Disease of Lignac</td>
<td>Rotten eggs odor</td>
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</tbody>
</table>
A. Lungs

Pathologies of the lungs, including bronchiectasis (dilation of bronchi), lung abscesses, and necrotizing pulmonic neoplasias may cause unpleasant odors to be exhaled (Mazzone, 2008).

B. Gastro Intestinal

It is very unfortunate that most patients and physicians still think halitosis originates in the stomach (Bollen & Beikler, 2012). The stomach is only responsible for halitosis in less than 0.5% of all cases (Bollen & Beikler, 2012). Therefore, even though some have asserted there is a possible link between Gastroesophageal Reflux Disease and halitosis (Struch et al., 2008), the mechanism by which acid-reflux causes halitosis has not been elucidated. Two extremely rare conditions of the esophagus can cause bad breath: bleeding and a Zenker’s Diverticulum (Stoeckli & Schmid, 2002). When the esophagus bleeds it causes a musty odor. A Zenker’s Diverticulum only happens in people older than 65 and its incidence is less than 0.1%. The Diverticulum is a small esophageal pouch that forms as a result of stretching.

In the stomach, infections with \textit{H. pylori} can cause peptic ulcers. Some studies suggest that these ulcers contribute to oral malodor. However, the results of the study were not statistically significant (Werdmuller et al., 2000).

Intestinal obstruction may cause fecal mouth odor. However, the condition is extremely rare and has other obvious symptoms (Stephenson & Rees, 1990).
C. Organ Failure

Kidney failure typically causes dry mouth and an increase in blood urea nitrogen. The urea nitrogen in blood then diffuses into the alveoli in the lungs and gets exhaled as air. The smell is an “ammonia” or “fishy” odor (Keles et al., 2011). Pancreatic failure also causes bad breath (Feller & Blignaut, 2005). (Exhaled through the lungs.)

Liver failure can result in bad breath, as waste products are directed to the lungs for elimination (Van den Velde et al., 2008). This causes “fetor hepaticus,” a fecal odor, to be exhaled.

D. Metabolic Disorders

Diabetic Ketoacidosis causes a typical odor when ketone bodies accumulate in the blood (Bollen & Beikler, 2012). It is a fruity, sweet odor. Also, Tyrosinemia is a hereditary metabolic disorder that causes cabbage breath (Bollen & Beikler, 2012). Patients who have the disease are unable to breakdown the amino acid tyrosine.

Trimethylaminuria is a hereditary metabolic disease that involves an enzymatic deficiency. It causes trimethylamine to accumulate in the blood and results in a fishy smell that is present in urine, sweat, and breath (Whittle et al., 2007). Trimethylamine is formed by bacteria in the colon and is usually converted to other nonvolatile compounds in the liver (Mackay et al., 2011). To alleviate the condition, antibiotics are taken to reduce the bacteria in the colon.
E. ENT Disorders

Chronic tonsillitis and sinusitis can cause significant malodor, as both are caused by bacteria known to produce volatile sulfur compounds (Mulwafu et al., 2006). Additionally, tonsilloliths, small calcified pieces of debris that attach to tonsils, can cause significant amounts of bad breath (Fletcher & Blair, 1988). Composed of dead cells and food matter, these pieces become food for oral bacteria which go on to produce volatile sulfur compounds—the primary bad breath culprits (Tsuneishi et al., 2006).

Tonsilloliths can go unnoticed, which results in slow but steady growth. Figure 1 shows a very large, white tonsillolith visible behind an individual’s tonsil. Most tonsilloliths detach and get swallowed way before reaching that size. A tonsillectomy, although drastic, eliminates tonsilloliths for good.

Figure 1. Large tonsillolith wedged behind a patient’s tonsil. (“Tonsillolith,” 2014).
**Intra-Oral Halitosis**

As stated earlier, approximately 90% of all cases of halitosis originate inside the oral cavity. Intra-oral halitosis can be further divided into Pathological Halitosis and Physiological Halitosis.

Pathological Halitosis is the result of overgrowth of bacteria due to periodontitis, an infection of the tissue surrounding the teeth (Morita & Wang, 2001). Daily brushing, flossing, and using mouthwash can reduce the bacteria that cause this infection, and reduce the smell as well. Gingivitis is a reversible form of periodontitis, but once serious tissue destruction begins, the disease is irreversible. The top photo in Figure 2 shows a typical presentation of gingivitis that is the result of poor oral hygiene.

![Figure 2. Gingivitis. Top photo shows plaque and gum inflammation, a mild form of periodontitis. Bottom shows healthy gums after cleaning and reversal of inflammation (“Gingivitis,” 2014).](image)

But for people who do maintain good oral hygiene, Halitosis can still occur. Its source is the dorsal (top) surface of the tongue (Roldán et al., 2003). This is
called Physiological Halitosis and is not the result of any disease. The tongue’s dorsal surface has a large surface area of 25cm² and is ideal for the growth of bacteria (Collins & Dawes, 1987). The papillary structure of the tongue (see Figure 3) is deeply grooved and ridged, allowing up to 100 bacterial cells to cling to one epithelial cell. This is four times the average of an epithelial cell on other surfaces of the mouth (Yaegaki & Coil, 2000). The deep ridges allow for an anaerobic environment to develop, one that is too deep for the flushing and cleansing effects of saliva (Steenberghe & Rosenberg, 1996). The tongue surface also promotes the entrapment of food debris and dead cells (Yaegaki & Sanada, 1992). Accumulation on the dorsum of the tongue results in a white or brown tongue-coating (see Figure 4). The halitosis smell comes from the bacterial byproducts inside the tongue coat (Van der Sleen et al., 2010).

Figure 3. The microscopic papillary structure of the tongue. (“Lingual papilla,” 2014). Figure 4. Tongue coat. (“White Coating on Tongue,” n.d.).
Some researchers have asserted that the morphology of the tongue promotes Halitosis (De Boever & Loesche, 1995). In other words, people who have excessively rough tongues are more likely to have Halitosis. However, other researchers have completely disputed those results (Gómez et al., 2001) (Quirynen et al., 1998). The difference in the results may be because of the different populations sampled (Roldán et al., 2003). The first researchers used a population of patients with more severe Halitosis.

It is interesting to note that patients who have Pathological Halitosis almost always have tongue coating too (Yasukawa et al., 2010). The list of periodontopathic bacteria and physiological malodor bacteria are very similar. Indeed, the quality of tongue coating has been attributed to factors that include periodontal status, age, salivary flow rate, and oral hygiene status (Ralph, 1987).

For data collection purposes, researchers have assigned the tongue a coating-index (Winkel et al., 2003). The Winkel Tongue Index is one of the most widely used. Winkel and his colleagues divided the tongue into six areas and independently scored each one from 0 (coating not present) to 1 (light coating present) and 2 (heavy coating). The final value of the Winkel Tongue index is obtained by adding all six individual scores.

One can imagine that the Dorsal Posterior section of the tongue, the surface of the tongue that is deepest in the oral cavity and least accessible to oral hygiene procedures, would probably have the most significant tongue coating. Allaker and his colleagues proved that this is exactly the case, with the total bacterial load
increasing when moving from anterior to posterior (Allaker et al., 2008). They also determined that the portion of the tongue known as the Dorsal Posterior Circumvallate Papillae (see Figure 5) is the part that most significantly contributes to malodor because it harbors the largest number of bacteria, the largest number of anaerobic gram negative bacteria, and the largest number of bacterial metabolic byproducts called volatile sulfur compounds (VSC). Their study also revealed that tooth brushing alone did not reduce the total numbers of tongue bacteria. Figure 6 summarizes Allaker’s findings.

Figure 5. Map of tongue showing Dorsal Posterior Circumvallate Papillae (Allaker et al., 2008).
With more scientific research being done on Halitosis, many pharmaceutical companies began manufacturing the tongue brush and the tongue scraper. The tongue brush is used to brush the tongue while the scraper is an instrument that a patient puts at the far back of his tongue and then drags forward while scraping all the plaque off of the tongue’s surface. Both have been proven to reduce tongue plaque (Quirynen et al., 2004). And the tongue scraper is especially good at providing visual proof of the plaque that accumulates at the far DPCP region of the tongue. However, it is possible that most patients don’t extend their tongue
scraper far enough into the DPCP because doing so strongly elicits the gagging reflex. Both the tongue scraper and the tongue brush have been proven to reduce oral malodor for a short time (Seemann et al., 2001). Neither have been proven to alone be remedies for halitosis (Menon & Coykendall, 1994). Both have been proven to slightly reduce the tongue’s bacterial load or to temporarily reduce it (Van der Sleen et al., 2010).

**Figure 7. Tongue scraper.** (“Tongue cleaner,” 2014)

Tongue plaque does not always result in Halitosis. In their 2007 publication, Tangerman and Winkel did not find a correlation between the degree of tongue coating and the intensity of the smell (Tangerman & Winkel, 2007). They concluded that the quality of the tongue coating was more important than the quantity. In an effort to learn more about tongue coating, researchers have tried to elucidate which bacteria in particular are responsible for the odor and how it is they make it.

As mentioned earlier, for intra-oral halitosis, the odor comes primarily from three bacterial byproducts known as VSC (Thorn & Greenman, 2012). They are Hydrogen Sulfide, Dimethyl Sulfide, and Methyl Mercaptan. These three...
Compounds are made primarily by gram-negative bacteria (Amou et al., 2014).

According to Bollen and Beikler, the total list of oral bacteria that have been implicated in the production of VSC includes 20 species names (Bollen & Beikler, 2012). See Table 3 for the complete list.

**Table 3. List of bacteria implicated in VSC production.** Adapted from (Persson et al., 1990) and (Bollen & Beikler, 2012).

<table>
<thead>
<tr>
<th>Volatile Sulfur Compound</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$S from cysteine</td>
<td><em>Peptostreptococcus anaerobius</em></td>
</tr>
<tr>
<td></td>
<td><em>Micros prevotii</em></td>
</tr>
<tr>
<td></td>
<td><em>Eubacterium limosum</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacteroides spp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Centipedia periodontii</em></td>
</tr>
<tr>
<td>H$_2$S from serum</td>
<td><em>Prevotella intermedia</em></td>
</tr>
<tr>
<td></td>
<td><em>Prevotella loescheii</em></td>
</tr>
<tr>
<td></td>
<td><em>Porphyromonas gingivalis</em> (BANA positive)</td>
</tr>
<tr>
<td></td>
<td><em>Treponema denticola</em> (BANA positive)</td>
</tr>
<tr>
<td></td>
<td><em>Selenomonas artermidis</em></td>
</tr>
<tr>
<td>CH$_3$SH from methionine</td>
<td><em>Fusobacterium nucleatum</em></td>
</tr>
<tr>
<td></td>
<td><em>Eubacterium spp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Bacteroides spp.</em></td>
</tr>
<tr>
<td></td>
<td><em>Fusobacterium periodonticum</em></td>
</tr>
<tr>
<td>CH$_3$SH from serum</td>
<td><em>Treponema denticola</em> (BANA positive)</td>
</tr>
<tr>
<td></td>
<td><em>Porphyromonas gingivalis</em> (BANA positive)</td>
</tr>
<tr>
<td></td>
<td><em>Porphyromonas endodontalis</em></td>
</tr>
<tr>
<td>Other</td>
<td><em>Prevotella melaninogenica</em></td>
</tr>
</tbody>
</table>
**Table:**

<table>
<thead>
<tr>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tanerella forsythensis</em></td>
</tr>
<tr>
<td><em>Eikenella corrodens</em></td>
</tr>
<tr>
<td><em>Solobacterium moorei</em></td>
</tr>
<tr>
<td><em>Treponema forsythensis</em></td>
</tr>
<tr>
<td><em>Centipeda periodontii</em></td>
</tr>
<tr>
<td><em>Atopobium parvulum</em></td>
</tr>
</tbody>
</table>

From the research conducted on the topic, one can make four general observations about Halitosis patients. (As you will read below, three of these observations have been disputed)

1. Halitosis patients always have more bacteria on their tongues, especially anaerobic gram-negative (Washio, Sato, Koseki, & Takahashi, 2005).
2. The Bacteria living on the tongue dorsum of the halitosis patient are much more diverse (Haraszthy et al., 2007) (Takeshita et al., 2010).

One paper claims the opposite, saying the bacterial community of the halitosis patient is the most conserved (Yang et al., 2013).

3. It can be difficult to directly implicate a species of bacteria for its involvement in VSC production. VSC production involves many players and can be very complicated (Yang et al., 2013). Considering that there are hundreds of uncultivable species of bacteria residing in the oral cavity, researchers have hesitated in naming culprits, even when identifying the species most commonly found on the tongues of Halitosis patients (Takeshita et al., 2012). Some scientists have fallen back on the “non-specific theory,” which says there are
multiple responsible groups and that different groups can substitute for each other (Takeshita et al., 2012).

On the contrary, *Fusobacterium nucleatum* has been directly implicated (Amou et al., 2014). *S. moorei* supposedly is present in one of five subjects without malodor and 3 of 6 with malodour and is therefore directly responsible (Haraszthy et al., 2007). Another paper directly implicated *Porphyromonas gingivalis*, *P. endodontalis*, and *F. nucleatum* but then holds back and says they might be silent observers that simply prefer living in an environment in which much VSC is produced (Takeshita et al., 2012). Other studies implicated specific taxa like *Prevotella* and *Leptotricia* (Yang et al., 2013) or *Veillonella, Actinomyces* and *Prevotella* (Washio et al., 2005).

Takeshita et al proposed that every person’s oral bacteria can be grouped into one of four bacterial-community profiles. They identified two of those communities as conducive to Halitosis, and mentioned which bacteria were most likely to be present (Takeshita et al., 2010).

4. The gram-positive bacteria, *Streptococcus salivarius*, is generally absent or present in extremely small numbers on the tongue dorsum of the halitosis patient (Allaker et al., 2008). And *Streptococcus salivarius* always makes up a sizeable fraction of the healthy normal tongue flora (Kazor et al., 2003).

On the contrary, another group of papers (see below) implicate *S. salivarius* as an indirect producer of VSC.

The primary food sources for oral bacteria are dead epithelial cells and
glycosylated saliva proteins (Yoneda et al., 2010). The gram-negative bacteria that produce VSC metabolize protein to obtain energy (Takehara et al., 2010). The gram-positive bacteria live by metabolizing the sugars on glycosylated proteins (N. Sterer & Rosenberg, 2006). Thus the bacteria in the oral cavity work together: the gram-positive bacteria remove the sugars and make the protein accessible to the gram-negative bacteria (Bradshaw et al., 1994). According to Sterer et al, the gram-positive bacteria are usually on the outer surfaces of the plaque biofilm, while the gram-negative are deeper inside (Sterer et al., 2009). Also, VSC levels have been known to increase as the carbohydrate content of salivary glycoproteins decreased (Takehara et al., 2010).

Many enzymes are responsible for the deglycosylation (Takehara et al., 2010). And studies must be done on all of these enzymes in order to provide a complete picture of the processes that allows VSC to be produced. Researchers have focused on β-Galactosidase as a model enzyme responsible for protein deglycosylation (Masuo et al., 2012).

Yoneda et al showed that there is a positive correlation between β-Galactosidase activity and thickness of tongue coating (Yoneda et al., 2010). Masuo et al showed that there is a positive correlation between β-galactosidase activity and intensity of oral malodour (Masuo et al., 2012). And β-Galactosidase activity has also been found to be positively correlated with amounts of total bacteria, *Fusobacterium nucleatum*, and *Streptococcus salivarius* (Masuo et al., 2012). Sterer and Rosenberg proved that *Streptococcus salivarius* (a gram-positive...
bacteria that displays β-Galactosidase activity) facilitates VSC production for the gram-negative *P. gingivalis* (N. Sterer & Rosenberg, 2006).

This creates a contradiction in the scientific literature. On one hand, researchers state that having more *S. salivarius* makes breath smell better. And on the other hand, *S. salivarius* would be contributing to malodor.

**VSC: Structure and Function**

Table 4 summarizes all compounds known to contribute to oral malodour. These can be detected by gas chromatography (Steenberghe et al., 1996).

The most important ones are the VSC (Thorn & Greenman, 2012): hydrogen sulfide (H\_2S), methyl mercaptan (CH\_3SH) and dimethyl sulfide (CH\_3SCH\_3). They are considered “volatile” because they are gaseous in nature, and can easily get exhaled as breath, even at room temperature (“bad_breath/t3/causes_of_halitosis.htm,” n.d.). These three will be discussed in detail below.

**Table 4. Total list of compounds that contribute to Halitosis.** Adapted from (Bollen & Beikler, 2012; Goldberg et al., 1994; Greenman et al., 2004; Steenberghe et al., 1996).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatile sulfur compounds</td>
<td>Methyl mercaptan: CH_3SH</td>
</tr>
<tr>
<td></td>
<td>Hydrogen sulfide: H_2S</td>
</tr>
<tr>
<td></td>
<td>Dimethyl sulfide: (CH_3)_2S</td>
</tr>
<tr>
<td>Diamines</td>
<td>Putrescine: NH_2(CH_2)_4NH_2</td>
</tr>
<tr>
<td></td>
<td>Cadaverine: NH_2(CH_2)_3NH_2</td>
</tr>
<tr>
<td></td>
<td>Butyric acid: CH_3CH_2CH_2COOH</td>
</tr>
<tr>
<td>Category</td>
<td>Compound</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>CH₃CH₂COOH</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>C₅H₁₀O₂</td>
</tr>
<tr>
<td>Phenyl compounds</td>
<td>Indole: C₈H₇N</td>
</tr>
<tr>
<td></td>
<td>Skatole: C₉H₁₀N</td>
</tr>
<tr>
<td></td>
<td>Pyridine: C₅H₅N</td>
</tr>
<tr>
<td>Alcohols</td>
<td>1-propoxy-2-propanol</td>
</tr>
<tr>
<td>Alkalines</td>
<td>2-methyl-propane</td>
</tr>
<tr>
<td>Nitrogen-containing compounds</td>
<td>Urea: (NH₂)₂CO</td>
</tr>
<tr>
<td></td>
<td>Ammonia: NH₃</td>
</tr>
</tbody>
</table>

Figure 8 summarizes the events that lead to bacterial production of VSC. First bacteria secrete peptidases that degrade protein in the nearby environment (Yoneda et al., 2010). The bacteria then absorb the amino acids for use as a fuel source ("bad_breath/t3-causes_of_halitosis.htm," n.d.). For the three VSC, it is important to note that they are made from sulfur-containing amino acids (Riggio et al., 2008). And that each VSC has a specific enzyme that produces it (Thorn & Greenman, 2012). The VSC are then released as a byproduct of bacteria metabolism (Washio et al., 2005).
Figure 8. Overview of VSC production. (Thorn & Greenman, 2012).

A. Methyl mercaptan CH$_3$SH

![Chemical structure of Methyl mercaptan](image)

Figure 9. Chemical structure of Methyl mercaptan. ("Methanethiol," 2014).

Inside the bacterial cell, methyl mercaptan is made from the amino acid methionine (Yoshimura et al., 2000). Recent data suggests patients with pathological Halitosis are much more likely to have breath that smells like methyl
mercaptan (Nakano et al., 2002). However, considering that methyl mercaptan has a recognition threshold that is $1/30$ that of hydrogen sulfide and a threshold of objectionability that is $1/8$ that of hydrogen sulfide, it is likely that all Halitosis patients have breath that smells like methyl mercaptan (Tangerman & Winkel, 2007). This is despite the fact that in most Halitosis patients the concentration of hydrogen sulfide is almost twice as high as that of methyl mercaptan (Tangerman & Winkel, 2007).

The objectionability threshold for methyl mercaptan and hydrogen sulfide are 0.5nmol/l and 4nmol/l respectively (Tonzetich, 1977).

Methyl mercaptan is a colorless and extremely flammable gas that has a disagreeable smell similar to that of rotten cabbage (“CDC-Methyl mercaptan,” n.d.). It occurs naturally in onions and garlic and other vegetables (Cai et al., 1995). It is used to produce pesticides, fungicides, jet fuel and plastics and in the synthesis of methionine (“ATSDR - Toxic Substances - Methyl Mercaptan,” n.d.). It is also used as a gas odorant for natural gas, propane and butane (“Making scents of it all | New York Post,” n.d.).

B. Hydrogen Sulfide $\text{H}_2\text{S}$
Hydrogen sulfide is a colorless gas with an extremely foul odor of rotten eggs; it is heavier than air, very poisonous, corrosive, flammable and explosive ( "CDC - Hydrogen sulfide," n.d., "ICSC 0165 - HYDROGEN SULFIDE," n.d.). When cysteine gets degraded to make Hydrogen sulfide, the sulfhydryl anion is also made and it is a strong reducing agent that drops the redox potential on the tongue biofilm. This promotes the growth of anaerobic bacteria and activates thiol-dependent enzymes like proteases and the same cysteine-desulfhydrase that produces hydrogen sulfide in the first place (Thorn & Greenman, 2012).

C. Dimethyl Sulfide (CH₃)₂S

Dimethyl sulfide has a characteristic smell similar to boiling cabbage ( "dimethyl sulfide - PubChem," n.d.). Although many studies consider it an oral VSC, more recent research seems to indicate that dimethyl sulfide plays a much more important role in extra-oral Halitosis (Tangeman & Winkel, 2007). (I.E a metabolic problem that releases odor through the blood and lungs)
Four additional compounds known to contribute to bad breath are cadaverine, putrescine, indole and skatole, which are formed from lysine, arginine, tryptophan, and tryptophan respectively (“bad_breath/t3_causes_of_halitosis.htm,” n.d.). Cadaverine is the smell associated with corpses. Putrescine is the smell of decaying meat. Skatole and indole are both found naturally in fecal matter and give off the characteristic fecal smell. Indole at high concentrations smells like fecal matter (and flowery at low concentrations).

Goldberg et al proved that cadaverine does significantly contribute to malodor and that its effects can be as significant as the VSCs (Goldberg et al., 1994). However, more recent research (Cooke et al., 2003) proves that saliva samples always contain more putrescine than cadaverine. Indole came in third, and skatole (3-methylindole) was almost always absent, but could be found in tongue plaque. All four compounds were very high upon waking and then significantly decreased after regular morning brushing with sodium fluoride, only to steadily rise again until lunch. The lunch meal was also proven to decrease the levels of the compounds.

Putrescine is found at higher concentrations than cadaverine because its substrates (arginine and ornithene) are more readily available in saliva than lysine.

The amines cadaverine and putrescine are formed by the bacterial process of putrefaction.
Why some have Halitosis and others don’t

It is not known exactly why some people develop chronic physiological malodor. In a study, Washio et al concluded that people who have physiological malodor are suffering from a drastically increased number of VSC-producing bacteria on the tongue dorsum (Washio et al., 2005). The study suggests that it may be a quantitative rather than a qualitative problem. But the study did not clarify why some people who maintain good oral hygiene would be able to maintain such large numbers of bacteria on the surface of their tongues. It is clear that other factors must be at work.

Every person has a unique oral microbiome. This microbiome is unique enough to be used for forensic identification (Leake, 2013). Throughout adolescence and adulthood, this microbiome is remarkably stable (Costello et al., 2009). Snapshots of a person’s oral microbiome at ages 30 and 40 will not necessarily be much different than at ages 30 and 31. However, it is during infancy and childhood that the microbiome undergoes remarkable changes (Könönen, 2000). Many factors contribute to the development of a final, stable oral ecosystem. Scientists are in dispute over what those factors are. They are grouped below into the two broad categories of “Genetics” and “Environment.”

1. Genetics

Nasidze et al concluded that people from different corners of the globe do not have significantly different oral bacterial populations (Nasidze et al., 2009). They surveyed 120 people from 12 worldwide locations and determined that location
and environment don’t affect which bacteria will be found in the mouth. They instead proposed that the genetic makeup of the host is the primary determinant.

A. Adhesins

The oral cavity contains over 700 species of bacteria (Parahitiyawa et al., 2010). Only about 400 of them can be cultured using traditional bacterial culturing techniques. Every surface of the mouth is covered in bacteria (Aas et al., 2005). Generally, these bacteria don’t cause any harm. Their most important function is to prevent other harmful and parasitic bacteria from taking root in the mouth and causing disease.

The oral epithelium of the mouth contains receptors for bacterial adhesins, allowing the bacteria to attach (Kolenbrander & London, 1993). The bacteria also have receptors on their surfaces which allow additional layers of bacteria to add on. This process is at the basis of the formation of dental plaque.

The receptors on the oral epithelium are proteins translated from the host’s DNA, thus contributing a genetic component to the mouth’s bacterial microbiome.

Figure 12 shows the various bacterial species that form plaque on the surface of teeth. Although the surface of the tongue has a unique set of receptors, some key bacteria like *F. nucleatum* appear to settle on both surfaces. Studies need to be done on the tongue surface to help identify the early and late colonizers.
B. Salivary proteins

While the bacteria living in the human mouth do get a meal every time you do, their primary source of food comes from saliva (Takehara et al., 2010). Saliva is...
rich in proteins and it is these proteins that provide nutrition for the resident bacteria (Bradshaw et al., 1994). Ingested food is only present in the mouth for short periods of times, and thus bacteria must get their food from elsewhere. Salivary proteins contain high levels of mucins, which are covered in carbohydrate (Wickström & Svensäter, 2008). Every person has unique mucins and unique carbohydrate attachments to the mucins (Helmerhorst & Oppenheim, 2007). Bacteria settle in the oral cavity depending on their ability to feast off of these mucins (Oppenheim et al., 2007). While no bacteria alone can degrade an entire mucin, many bacteria working together certainly can (Takehara et al., 2013). And it is the identity of the mucin, in addition to the bacteria already present in the oral cavity that determine whether or not a species of bacteria will settle in the mouth and become one of the resident bacteria. Since saliva proteins are translated from the host’s DNA, this is a genetic component that affects the species of bacteria which settle in the mouth.

C. Salivary flow

Severely decreased salivary flow increases malodor (Koshimune et al., 2003). This can be attributed to an increase in gram-negative bacteria and to an accumulation of debris on the surface of the tongue (Almståhl & Wikström, 1999; Kleinberg et al., 2002). However it is not known if the majority of halitosis patients have a slightly decreased flow of saliva. When saliva flow decreases, the concentration of salivary proteins increases, which gives more nutrition to the
VSC producing bacteria (Sopapornamorn et al., 2007; Takehara et al., 2010; van den Broek et al, 2007).

However, one group of scientists disagree and point out that salivary flow is usually increased in malodor patients, and this increase in saliva output is what potentiates the malodor by giving the bacteria more nutritional lifeline (Hinode et al., 2003). (The latter opinion does not think decreased salivary flow increases salivary protein concentration).

D. Antibodies

(Hinode et al., 2003) have tried to find out the status of the immune system in the malodor patient. S-IgA is the primary antibody of the oral cavity and it activates the complement system against invading bacteria. It also promotes bacterial clumping for phagocytosis.

Patients with only slight tongue coating were found to have significantly higher concentrations of IgA. For patients with severe malodor and significant tongue coating, the IgA recognized very strongly components of *Fusobacterium nucleatum* and *P. intermedia*, two VSC producers.

Hinode and colleagues concluded that IgA may play a protective role against the accumulation of tongue plaque.

E. Tongue coating

In a study that tried to examine whether physiological halitosis has a genetic component, researchers compared if monozygotic twins were more likely to have bad breath than dizygotic twins (Bretz et al., 2011). They concluded that if one
monozygotic twin had significant tongue coating, the other had a very high chance of having the same condition. And this was much more likely for monozygotic than dizygotic twins. The concordance rates were 67% and 11%, respectively. The study concluded that there must be some genetic component that allows bacterial mats to grow on the tongues of certain individuals.

2. The environment

While genetics may play a massive role, the environment can certainly point genetics in a certain direction. Below are some environmental situations that can influence which bacteria settle in the human oral cavity.

A. Environmental stresses

Sjögren's syndrome is a disease in which the immune system destroys the salivary glands. Studies have shown that this disease changes the composition of the oral microbiome (MacFarlane & Mason, 1974). Chronic dehydration can be known to have a similar effect (Almståhl & Wikström, 1999). Studies have shown that environmental stress factors can change the bacterial composition of the mouth (MacFarlane & Mason, 1974; Marsh, 2006). These studies have discussed periodontitis and cavies, claiming that these disease are really mini-ecological catastrophes in the mouth. It is unknown what stresses, besides Sjogren’s syndrome and chronic dehydration, can cause the oral microbiome to change into an environment that produces malodour.

B. Family members.
It has been proven that mothers can infect their infant or toddler with the bacteria that cause cavities (Li et al., 2007). And family members can acquire periodontal pathogens from one another (Van Winkelhoff & Boutaga, 2005). And in general, family members share oral bacteria species with one another and even with their pets (Song et al., 2013). Similar studies are warranted in regards to the halitosis-causing bacteria; this could shed some light on the development of the disease.

C. Taking antibiotics.

It is not known what effect taking an antibiotic can have on the resident oral flora of the mouth. During childhood, when the oral microbiome is still being developed, an antibiotic can have a much more significant effect than during adulthood.

**How to objectively measure breath**

Bad breath can be detected organoleptically (Tangeman & Winkel, 2008). This entails having at least two trained judges smell the breath and identify its severity. Table 5 shows the Rosenberg and McCulloch scale, the most recognized scale for determining the severity of breath (Vandekerckhove et al., 2009).

**Table 5. Rosenberg and McCulloch scale for breath.** (Rosenberg & McCulloch, 1992)

<table>
<thead>
<tr>
<th>Organoleptic Breath Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No detectable odor</td>
</tr>
<tr>
<td>1</td>
<td>Hardly detectable odor</td>
</tr>
<tr>
<td>2</td>
<td>Light odor</td>
</tr>
<tr>
<td>3</td>
<td>Moderate odor</td>
</tr>
</tbody>
</table>
Traditionally, organoleptic testing has been the gold standard for diagnosing bad breath (Vandekerckhove et al., 2009). However, gas chromatography is the most effective because it provides the most sensitive and detailed analysis of breath (Murata et al., 2002). The equipment is very expensive and requires trained personnel but can identify every chemical compound in exhaled breath (Furne et al., 2002).

Two other machines have been developed specifically to test for bad breath. The first is the Halimeter, developed and first used in 1990 (“The Halimeter® — Measure Bad Breath Scientifically,” n.d.). While this machine made testing for bad breath simple and quick, more recent studies have found its competitor, the OralChroma, a much better substitute (Tangerman & Winkel, 2008). The Halimeter needs to be recalibrated much more frequently than the OralChroma (Vandekerckhove et al., 2009). And the Halimeter is almost completely ineffective at measuring for dimethyl sulfide, a significant VSC (Tangerman & Winkel, 2008).

What made the Halimeter very popular, though, was its small screen that displays a rapid reading.
When the OralChroma was introduced in the US and Canada in 2003, Tangerman & Winkel noticed that patients sometimes differed in the composition of their “mouth” breath and their “nose” breath (see Figure 14). Some patients were found to have no VSC coming from their mouths, rather, only from their nose, which supplied air directly from the lungs (graphs C and D). While other patients had VSC only in their mouth breath and not in their nose breath (graphs A and B).

Using the OralChroma, Tangerman and Winkel concluded that the primary VSC coming from the mouth and tongue were methyl mercaptan and hydrogen sulfide. Hydrogen sulfide being the more dominant of the two. And that dimethyl sulfide was sometimes found only in patients’ nose breath. These patients
became known as Extra-oral Halitosis patients. For some metabolic reason, their blood contained dimethyl sulfide, which unlike hydrogen sulfide and methyl mercaptan is stable in blood, and can diffuse into the lungs and out through the nose (Blom & Tangerman, 1988).

Figure 14. Difference between intra-oral and extra-oral halitosis. Graphs (A) and (B) are mouth breath and nose breath of a patient with oral halitosis. (C) and (D) are mouth breath and nose breath of a patient with extra-oral halitosis. Peak 1 is H₂S. 2 is methyl mercaptan. 3 is dimethyl sulfide. (Tangerman & Winkel, 2007)

The OralChroma (see Figure 15) costs $6000 dollars and is considered a portable Gas Chromatograph (Tangerman & Winkel, 2008). It takes approximately 8 minutes to display its reading and has threshold values of 112, 26, and 8 ppb for hydrogen sulfide, methyl mercaptan, and dimethyl sulfide respectively. It needs to be attached to a computer and has its own software. Figure 16 shows the kind of bar graphs that are produced for the three VSCs when the Oralchroma produces its reading.
Figure 15. The OralChroma, a portable gas chromatograph. ("Oral Chroma, a halitosis measuring device; Inquiry," n.d.)

Figure 16. Computer Display of the OralChroma software. Measurements for the three VSC are shown in the graph. ("Dental tips/Dentistry in Shanghai," n.d.)

It is not known how many dentists across the US have Halimeters and Oralchromas. Or how much they charge for a bad breath consultation or
examination. More studies need to be done to determine just how knowledgeable
American Physicians and Dentists are in regards to the true causes of Halitosis.
A first-of-its-kind study published in 2014 by Oppliger et al compared the
knowledge of 750 Dentists and hygienists in France, Switzerland and Germany
(Oppliger et al., 2014). Hygienists in general were found to be most aware of the
true causes of Halitosis while French Dentists were the least. French Dentists
were also least likely to recommend use of a tongue scraper.
A bad breath consultation at a New Zealand clinic that involves having a dentist
test one’s breath with an OralChroma costs $121. A more thorough bad breath
consultation that includes an OralChroma check in addition to saliva, gums, and
dietary analysis costs $242 ("New Zealand Fresh Breath Clinic | Bad Breath
Treatment | North Shore | Milford Dentists," n.d.).

Rinses and Toothpastes
Most tooth pastes contain sodium lauryl sulfate, a foaming agent. While this
ingredient may seem like a necessary component of toothpastes, it oftentimes
exacerbates the problem for halitosis patients who experience severe dry-mouth
after using traditional toothpaste ("SLS Free Toothpaste," n.d.). The same
applies for mouthwash. The majority of mouthwashes give the halitosis patient
dry-mouth and only exacerbate the problem. Any freshening effect they have is
only a momentary masking effect (Sterer & Rubinstein, 2006). While these
products may do an excellent job removing the plaque that accumulates on the
surface of teeth, they are not meant to decrease the bacterial load that forms on the dorsum of the tongue or to neutralize the VSCs. Because the primary habitat of the bacteria is the dorsum of the tongue, and gargling is the best way of reaching that area, mouthwashes have emerged as the primary way of combatting halitosis. Table 6 shows the anti-halitosis mouthwashes that have been developed over the years, and the various countries in which they were manufactured. Clinicians operating a popular fresh breath clinic in Belgium have stated that most patients, when instructed on which mouthwashes were the right ones to use, did see their bad breath symptoms improve (Quirynen et al., 2009). The primary antibacterial components are chlorhexidine, metal salts, chlorine dioxide, and CPC. (See below for more detail).

Table 6. Rinses for Halitosis Patients.

<table>
<thead>
<tr>
<th>Name of Mouthwash</th>
<th>Active Ingredient</th>
<th>Country of Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breathrx</td>
<td>Zinc, CPC</td>
<td>USA</td>
</tr>
<tr>
<td>Therabreath</td>
<td>ClO2</td>
<td>USA</td>
</tr>
<tr>
<td>Oxyfresh</td>
<td>Zinc, ClO2</td>
<td>USA</td>
</tr>
<tr>
<td>Profresh</td>
<td>ClO2</td>
<td>USA</td>
</tr>
<tr>
<td>Tom’s of Maine</td>
<td>Zinc</td>
<td>USA</td>
</tr>
<tr>
<td>Smartmouth</td>
<td>Zinc</td>
<td>USA</td>
</tr>
<tr>
<td>Halita</td>
<td>CHX, CPC, Zinc</td>
<td>Spain</td>
</tr>
<tr>
<td>Perio-Aid</td>
<td>CHX, CPC</td>
<td>Spain</td>
</tr>
<tr>
<td>Corsodyl</td>
<td>CHX</td>
<td>Belgium</td>
</tr>
</tbody>
</table>
1. Chlorhexidine

While searching for an anti-malaria drug in 1954, researchers working for Imperial Chemical Industries discovered chlorhexidine (Lim & Kam, 2008). They found it to be an extremely effective anti-microbial. It was not until 1976 that chlorhexidine was used for oral care (Puig Silla et al., 2008). Chemically, it is a strong base and has cationic properties (Puig Silla et al., 2008). Chlorhexidine solutions are odorless, colorless, and extremely bitter (Lim & Kam, 2008).

![Chemical structure of Chlorhexidine](image)

**Figure 17. Chemical structure of Chlorhexidine.** (“Chlorhexidine Facts: The Molecule,” n.d.)

Chlorhexidine is effective against gram-positive and gram-negative bacteria and
works by disrupting the cell membrane (Genuit et al., 2001). Within 30 seconds, it can kill nearly 100% of bacteria (Genuit et al., 2001).

Before disrupting the cell membrane, chlorhexidine, a positively charged molecule, binds to the negatively charged phospholipids on the bacterial cell walls and interferes with osmosis (Silla et al., 2008). Once the cell membrane becomes damaged, cytoplasm leakage results in death of the bacterial cell (McDonnell & Russell, 1999). In low concentrations, chlorhexidine damages cell walls; in high concentrations it causes cytoplasm to solidify (McDonnell & Russell, 1999). Thus, the molecule is both bacteriostatic and bactericidal. Chlorhexidine also binds to the epithelium of the mouth and is slowly released to continue to kill bacteria over time (“Chlorhexidine Facts: The Molecule,” n.d.). This makes chlorhexidine the gold standard for antiplaque and antigingivitis medications (Mathur et al., 2011). Even though chlorhexidine has been used for 60 years, there has not been any reported bacterial resistance to this drug (Genuit et al., 2001).

In the US, chlorhexidine rinses are prescription drugs. The two most popular ones are Peridex and Periogaurd. However, in other countries, mouthwashes have been made that have chlorhexidine in them. These include Halita, Corsodyl, and Perio-aid. Chlorhexidine is known to cause staining of the teeth and to alter taste perception (Herrera, 2013). However, newer rinses now claim to have an anti-discoloration system (Bernardi et al., 2004).
2. Metal Salts

Zinc, fluoride, copper, silver, and tin ions have all been proven to improve halitosis (Wåler, 1997). Zinc is considered the most effective (Wåler, 1997). It is assumed that these ions, because of their affinity for sulfur, oxidize the VSCs or their precursors into non-volatile substances (Ng & Tonzetich, 1984). Even though zinc is the most effective, it is not the element that has the highest affinity for sulfur. Zinc’s efficacy has to do with its ability to linger in the oral cavity and for it to remain potent (unoxidized) for longer periods of time (Wåler, 1997). Zinc is also involved in many regulatory pathways in bacterial cells (He et al,
Increasing the amount of zinc in the oral cavity will inhibit bacterial metabolism. Zinc has been proven to decrease the amino acid and sugar catabolism of *Fusobacterium nucleatum* and *Prevotella intermedia* (Choi et al., 2010) (Sheng et al., 2005).

3. CPC

![Chemical structure of cetylpyridinium chloride](image)

**Figure 19. Chemical structure of cetylpyridinium chloride, an anti-microbial agent.** ("Cetylpyridinium chloride," 2014)

Cetylpyridinium chloride is oftentimes an active ingredient in mouthrinses along with zinc and chlorhexidine (Saad et al., 2011). Recent studies, trying to pinpoint its activity, have concluded that it inhibits the growth and VSC production of *P. gingivalis* and *Fusobacterium nucleatum* (Liu et al., 2013). It also inhibits the two genes which express the two enzymes responsible for making the two most potent VSC, methyl mercaptan and hydrogen sulfide (Liu et al., 2013).

CPC has a long history of being safe for oral hygiene. It has also demonstrated plaque-reducing benefits (Sreenivasan et al., 2013).

4. Chlorine Dioxide ClO₂

Is a powerful and potent oxidizing agent, commonly used as a bleaching agent.
(Lynch et al., 1997). Already known to be bactericidal, it has been proven to
directly eliminate VSC by oxidizing them into neutral compounds (Shinada et al.,
2010). By doing so, it also helps consume the amino acids which produce VSC in
the first place (Shinada et al., 2010).

**Probiotics**

Oral rinses only provide a temporary fix (Saad et al., 2011). It is not long before
the VSC-producing bacteria repopulate the tongue and begin producing VSCs
again (Blom et al., 2012). Many researchers began searching for a more long
term solution. One that involved introducing friendlier bacteria into the oral cavity
(Franklin, 2013).

Probiotics are defined by the world health organization as live microorganisms,
which, when administered in adequate amounts confer a health benefit on the
have been used in the Colon to outcompete disease-causing bacteria (Taibi &
Comelli, 2014).

Kazor and coworkers (2003) discovered that *Streptococcus salivarius* is missing
in halitosis patients(Kazor et al., 2003). And that *S. salivarius* is much more likely
to be found in healthy individuals.

*Streptococcus salivarius*, a natural and early inhabitant of the oral cavity, does
not produce VSCs and is not known to cause any infections (Park et al., 2005) (Wescombe et al., 2009).

It also produces two bacteriocins (A and B) that kill other bacteria (Wescombe, 2010.). When the plasmid containing the bacteriocin genetic code is taken up by other bacteria, the manufacture of the bacteriocins can be greatly amplified (Wescombe et al., 2006) (Burton et al., 2005). Salivaricin A is bacteriostatic, while salivaricin B is bactericidal (Wescombe et al., 2009).

*Streptococcus salivarius* was first used as a probiotic to promote throat health (Sanders, 1982). Several children who rarely experienced sore-throats were found to have an abundance of *Streptococcus salivarius* in their oral cavities. Presumably, the *Streptococcus salivarius* was preventing *Streptococcus pyogenes* from causing throat infections. Researchers discovered the bacteriocins and that *Streptococcus salivarius* would preferentially bind to the epithelial cells of the human mouth, preventing *Streptococcus pyogenes* from taking root.

The use of *Streptococcus salivarius* as a safe probiotic for throat health facilitated its use for halitosis patients (Burton et al., 2005). It is trademarked under the name of Blis K12, and sold by several pharmaceutical companies, like NOW Foods (see Figure 21) (BLISK12.com, n.d.). As the bottom of Figure 20 indicates, Blis K12 has not yet been approved by the FDA.
Patients who use *Streptococcus salivarius* as a probiotic are supposed to use a chlorhexidine mouth rinse before-hand. According to Burton et al, this clears the palate of many of the VSC producing bacteria and makes the probiotic much more effective, allowing patients to maintain reduced levels of VSC for at least two weeks (Burton et al., 2005).

Another study (Burton et al., 2006) concluded that reduced VSC levels always correlated with increased amounts of *Streptococcus salivarius* K12 in the saliva of patients using the probiotic lozenge. This was determined using Denaturing Gradient Gel-Electrophoresis of 16s rRNA samples from bacterial DNA.
Figure 22. Gel-Electrophoresis of bacterial DNA. (A) prior to treatment. (B) Eight patients with decreased VSC levels always had a more predominant band representing the presence of *S. salivarius*. (C) Five patients with no decrease in VSC levels. (Burton et al., 2006)

*Streptococcus salivarius* inhibits the growth of several bacteria known to contribute to Halitosis (Wescome et al., 2012). These were tested in vitro and include *Streptococcus anginosis*, *Eubacterium saburreum*, *Micromonas micros*, *Porphyromonas gingivalis*, *Prevotella sp intermedia*, and black pigmented bacteria (Burton et al., 2006). Figure 23 shows the inhibition zone of *S. salivarius* against nine halitosis-associated gram-positive bacteria (Masdea et al., 2012). And one of them, *S. moorei* was not found to develop resistance to the bacteriocins that *Streptococcus salivarius* produces.
Halitosis can have severe social consequences that force a person to undergo lifestyle changes (Zalewska et al., 2012). Excess brushing, rinsing, and gum chewing are common (Uguru et al, 2011). Sufferers avoid onion, garlic, and protein-rich food at all cost (Uguru et al., 2011). Many also tend to consume lots of sugary foods in an effort to mask the smell (Bretz et al., 2011).

De Jonghe and coworkers have reported that halitosis patients almost always tend to avoid getting near other people (de Jongh et al, 2013). Halitosis can directly lead to depression as patients report failed careers, relationships, and a drastic increase in isolation (Uguru et al., 2011). McKeown et al (2003) points out that severe halitosis is a disability (McKeown, 2003).

Figure 23. Graph showing inhibition zone of *S. salivarius* against bacteria associated with halitosis. (Masdea et al., 2012)
Sugiyama and coworkers (2011) reported that halitosis patients exhibited high levels of kindness and patience and seemed to be holding themselves back (Sugiyama et al., 2011). The same study asserts that the personality types of halitosis patients don’t follow the same distribution as a sample of normal individuals.

A fresh breath clinic in Belgium that had over 2000 patients reports that many of its patients had been experiencing symptoms for years and that they had looked for help in different places (Quirynen et al., 2009). This clearly indicates that there is a general lack of awareness among dentist and doctors.

In the Belgian clinic, most patients only came for one consultation. Forty one percent returned for a follow up. Roughly 79% of patients were either cured or had experienced significant improvement, which indicates that genuine halitosis can almost always be resolved with the correct treatment.

There are two kinds of halitosis patients who don’t have true halitosis. Instead they are afflicted by pseudo-halitosis or halitophobia (Yaegaki & Coil, 2000). Pseudo-halitosis is when a person thinks he has halitosis but actually doesn’t (Campisi et al., 2011). Testing his breath with a Halimeter or OralChroma is usually enough to convince this patient that his breath smells fine. However, the halitophobia patient never gets convinced that his breath is fine (Zalewska et al., 2012). No amount of convincing by a dentist or doctor will get him to change his mind. This person is usually referred to psychiatric care, because of an underlying psychological problem. However some studies point out that the
halitophobia patient may have the ability to detect extremely small amounts of VSC on his tongue by retro-nasal olfaction (Falcão et al., 2012). (Quirynen et al., 2009) have put the number of pseudohalitosis/halitophobia patients at 16% of all halitosis patients. Another study indicated that the number is closer to 38.5% (Pham, 2013). Both think the number is rising because of increased media campaigns that promote fresh-breath products.
DISCUSSION

The fresh breath industry in the US is estimated to be worth three billion dollars ("Breath Mints," n.d.). Advertisements for chewing-gum and mouthwash, along with references in popular culture, have made bad breath into an extremely unacceptable social behavior. As many studies have pointed out, it is possible for someone to have Halitosis while maintaining excellent oral hygiene. This, however, does not stop halitosis patients from feeling severe embarrassment when symptoms arise.

Although the scientific literature has established which kinds of mouth rinses are the most effective for treating halitosis, there has not been as much agreement on what is causing the disease and what long term options are available for patients. The problem lies in the complexity of the oral microbiome. Until more bacterial species are identified and named, and their relationships with other bacteria are understood, a cure for this disease will remain elusive.

Streptococcus salivarius remains a point of contention. Some say it is the missing cure, others say it promotes VSC production. Further studies are needed to better establish its role.

In terms of their tongue bacteria, Halitosis patients and non-Halitosis individuals are more similar than they are different. It is possible that the halitosis and non-halitosis patients actually have the same bacteria on their tongues, but for some reason the VSC-producing bacteria exhibit an explosive population growth and
distort the numbers, making it appear as though the Halitosis patient has an entirely different microbiome (Washio et al., 2005).

A study should be done to analyze the tongue bacterial population of Halitosis patients soon after use of a chlorhexidine rinse, and that data should be compared to non-halitosis individuals.

The VSC producing bacteria have been identified, but it is unclear why they are present in such large numbers. Studies that blame the increased rate of protein deglycosylation by gram-positive bacteria are not seeing the bigger picture.

Clearly, gram-positive bacteria can be very beneficial, especially considering *S. salivarius* is able to function as a probiotic. Studies need to determine how non-Halitosis individuals keep their gram-negative bacteria under control, and what situations cause these gram-negative bacteria to grow in number.

An excellent place to start would be morning breath. Both halitosis and non-halitosis patients get it, and it’s caused by the same bacteria and VSCs, but for some reason morning breath fades for the non-halitosis individuals. While for the Halitosis patients, morning breath lasts all day long.
REFERENCES


Count/dp/B003P7YVTQ/ref=sr_1_1?ie=UTF8&qid=1402618311&sr=8-1&keywords=blis+k12+probiotic


with a sulfide detector vs. gas chromatography. *Journal of Dental Research, 81*(2), 140–143.


doi:10.3389/fgene.2013.00282


preparation containing the stable free radical species chlorine dioxide (ClO2.). *Free Radical Research, 26*(3), 209–234.


microbiota with one another and with their dogs. *eLife*, 2, e00458.
doi:10.7554/eLife.00458

doi:10.1016/j.tripleo.2006.08.023


doi:10.1177/154405910608501007


salivarius as model oral probiotics. *Future Microbiology, 4*(7), 819–835. doi:10.2217/fmb.09.61


CURRICULUM VITAE

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EDUCATION

BOSTON UNIVERSITY SCHOOL OF MEDICINE, Boston, MA
Masters of Science, Medicine, September 2014
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Relevant Coursework
Genetics, Physiology, Biochemistry, Histology, Pathology, Organic Chemistry, Business of Biotech, Public Speaking

Lab Skills
Chromatography, electrophoresis, assaying, animal dissection

Awards
Third place, Jerome A. Nachman Award (Journalism-writing contest)

CERTIFICATIONS

Emergency Medical Technician
EMS Training of Norwood, Norwood, MA
Learned to dress wounds, splint broken bones, obtain vitals, perform advanced CPR, maintain an airway, and deal with Diabetic, Cardiovascular, OB/GYN, and Head and Spine Emergencies. (5/13-9/13)
Nationally Certified on 9/19/13.

CLINICAL EXPERIENCE

Woonsocket Urgent Care, Woonsocket, RI
Assisted and shadowed Emergency Room Physician. Gathered patient-data and observed patient-doctor interactions. (6/12--9/12)

Steward Norwood Hospital, Norwood, MA
Observed hospital staff for compliance with Infection Control Department hand-washing guidelines. Published statistics in an effort to increase compliance. Aided nurses and doctors in Endoscopy, Emergency, Infection Control, and Transport Depts. (9/05--10/08)
Joslin Diabetes Clinic, Boston, MA
Assisted doctors and nurses in diabetes weight-loss study. Consolidated data for long term analysis. (10/07--12/07)

WORK EXPERIENCE

Walmart Supercenter, East Walpole, MA

Sunday School, ICNE, Sharon, MA

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Arabic Teacher - Taught 2nd and 3rd grade Arabic. (9/05--present)

Al-Noor Academy, Mansfield, MA
Science Teacher - Taught middle-school-level biology, physics and chemistry with emphasis on lab-work. (12/09--6/11)

COMMUNITY SERVICE

ICNE Youth Group, Sharon, MA
Director - Scheduled weekly-speaker for educational meetings. Held Public Speaking workshops. Trained Youth for leadership positions. Mentored teenagers in a campaign to decrease recreational drug use. Organized competitions and coached an interscholastic team. (10/10-8/12)