Chapter 35

Evidence-Based Control of Oral Malodor

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1. Introduction

Concern regarding halitosis is estimated to be the third most frequent reason for people to seek dental care, following tooth decay and periodontal disease [1]. Compared with tooth decay and periodontal disease, there are a diverse number of causes of halitosis. Table 1 shows a commonly used classification of halitosis [2 – 4]. Obvious bad breath is termed genuine halitosis, which is classified as physiological and pathological halitosis. Pathological halitosis is further sub-classified into halitosis as a result of oral and extra-oral causes. Physiological and oral pathological halitosis occur in the oral cavity, and comprise 85% or more of genuine halitosis [5, 6]. Physiological halitosis generally occurs at the time of waking or starving, and likely results from increased microbial metabolic activity that is aggravated by a physiological reduction in salivary flow, oral cleaning, and inadequate mouth cleaning before sleep or after eating [4]. Clinical causes of oral pathological halitosis include poor oral hygiene, tongue debris, periodontitis, inadequately fitted restorations, deep caries, endodontic lesions, ulceration, and low salivary flow [7 – 11]. The most common malodorous compounds that cause oral-derived malodor are volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH), which are associated with microbial amino acid metabolism [12, 13]. Halitosis derived from extra-oral causes is less common, but causes include respiratory disorders, gastrointestinal diseases, metabolic disorders, and drugs [2 – 4]. The smell of gases that have accumulated in organs during respiratory disorders and gastrointestinal diseases can be emitted directly from the oral cavity and nose. Malodorous components caused by some metabolic disorders and drugs circulate in the bloodstream and are exhaled in the breath after alveolar gas exchange. Components of extra-oral malodor include those due to disease, such as acetone in uncontrolled diabetes and trimethylamine in trimethylaminuria ("fish odor syndrome" [14]). Dimethyl sulfide (CH_3SCH_3), a VSC, is the main contributor to extra-oral or blood-borne halitosis via an as-yet-unknown metabolic disorder [15]. Some patients that complain of halitosis do not have bad breath. Although

pseudo-halitosis is not diagnosed as a psychiatric disorder, some patients with this condition exhibit neurotic tendencies more frequently than do patients with genuine halitosis [6]. Halitophobia is characterized by a patient's persistent belief that he or she has halitosis, despite reassurance, treatment, and counseling. Many patients with halitophobia have slight bad breath at their first visit to a dental clinic. However, the presence of a mental condition together with bad breath has been suggested in these individuals.

Classification (treatment needs)	Description
Genuine halitosis	Obvious malodor, and of an intensity beyond the socially acceptable level is perceived.
Physiological halitosis (TN-1)	Malodor arises through putrefactive processes within the oral cavity. No specific diseases or pathological conditions that could cause halitosis are found.
Pathological halitosis	
Oral (TN-1 and TN-2)	Halitosis caused by a disease or a pathological condition that causes malfunction of the oral tissues.
Extra-oral (TN-1 and TN-3)	Malodor that originates from a respiratory system, gastrointestinal tract, metabolic disorders, or drugs.
Pseudo-halitosis (TN-1 and TN-4)	No objective evidence of malodor, although the patient thinks they have it.
Halitophobia (TN-1 and TN-5)	The patient persists in believing they have halitosis despite reassurance, treatment, and counseling.

Table 1. Classification of halitosis [2-4].

All patients that complain of halitosis should receive an explanation of halitosis and instructions for oral hygiene (TN-1; Table 2) [16]. Further professional instruction, education, and reassurance are necessary for patients with pseudo-halitosis (TN-4). Professional cleaning and treatment of oral diseases are performed in patients with oral pathological halitosis (TN-2), and treatment and control of the systemic causative disease by a physician or medical specialist is provided for patients with extra-oral pathological halitosis (TN-3). Medical treatment by a psychological specialist is required for the treatment of halitophobia, regardless of the presence of bad breath (TN-5).

Category	Treatment regimen
TN-1	Explanation of halitosis and instructions for oral hygiene.
TN-2	Oral prophylaxis, professional cleaning, and treatment for oral diseases, particularly periodontal diseases.
TN-3	Referral to a physician or medial specialist.
TN-4	Explanation of the examination data, further professional instructions, education, and reassurance.
TN-5	Referral to a clinical psychologist, psychiatrist, or other psychological specialist.

Table 2. Treatment needs (TN) for halitosis [2, 16] useful for clinical dentists.

Most genuine halitosis occurs in the oral cavity, and is known as oral-derived malodor. As mentioned above, VSCs are produced during the metabolism of the sulfur-containing amino acids cysteine and methionine by bacteria [12, 13]. Gram-negative anaerobes in the oral cavity are important producers of VSCs. Periodontopathic bacteria isolated from subgingival plaques, such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*, generate significant amounts of H_2S and CH_3SH [17]. The genera *Veillonella*, *Actinomyces* and *Prevotella* are H_2S-producing normal inhabitants of the tongue coating [18]. *Solobacterium moorei* is present in the tongue dorsa of subjects with halitosis, specifically [19]. A recent investigation of the bacterial composition of saliva reported that high proportions of the genera *Neisseria*, *Fusobacterium*, *Porphyromonas*, and SR1 were present in patients with high H_2S and low CH_3SH, whereas high proportions of the genera *Prevotella*, *Veillonella*, *Atopobium*, *Megasphaera*, and *Selenomonas* were detected in patients with high CH_3SH and low H_2S [20]. The human oral cavity contains more than 500 bacterial species that interact both with each other and host tissues, suggesting that various bacteria might play roles in malodor production. The treatment strategy for oral-derived malodor is the acquisition of a normal microbiota, as well as reducing the numbers of bacteria. The prevention and treatment of oral malodor involve primarily the removal of any causative clinical conditions, predominantly via oral hygiene instructions and the treatment of oral diseases. Persistent malodor usually originates from the posterior dorsum of the tongue and/or oral/dental diseases, including periodontal diseases. Tongue cleaning and the treatment of periodontal diseases are effective for improving oral malodor [21, 22]. In addition, many products such as mouthwash, dentifrice, gel, gum, oil, tablets, and lozenges can play supporting roles in controlling oral malodor. Such products improve oral malodor by reducing bacterial load and/or nutrient availability, exerting anti-inflammatory effects, and converting VSCs into non-volatile substances. The active ingredients used for controlling oral malodor can be separated into chemical agents and naturally derived compounds. Examples of chemical agents include chlorhexidine, cetylpyridinium chloride, zinc chloride, triclosan, stannous fluoride, hydrogen peroxide, chlorine dioxide, and sodium fluoride. Naturally derived compounds can be sub-classified into natural botanical extracts (e.g., actinidine, hinokitiol, eucalyptus-extract, green tea, magnolia bark extract, and pericarp extract of garcinia mangostana L), salivary components (lactoferrin and lactoperoxidase), and probiotic bacteria (*Lactobacillus salivarius*, *Lactobacillus reuteri*, *Weissella cibaria*, and *Streptococcus salivarius*). In this chapter, these various approaches to the prevention and treatment of oral malodor are summarized.

2. Chemical agents

Chlorhexidine (CHX), cetylpyridinium chloride (CPC), triclosan, zinc ions (Zn^{2+}), and chlorine dioxide (ClO_2) are all known to inhibit oral malodor [23, 24]. In many cases, these active ingredients have been used in mouthwashes and dentifrices, both individually and in combinations. CHX digluconate has been used most frequently to treat oral cavities as an active ingredient in mouthwash that is designed to reduce dental plaque and oral bacteria. CHX is used in mouthwashes at 0.12% or 0.2%, and a previous study revealed that these two concen-

trations of CHX had an identical effect on gingival inflammation [25]. Young et al. [26] evaluated the inhibitory effects of CHX, CPC, and Zn^{2+}on VSC production. Data revealed that 0.2% CHX and 1% Zn^{2+}exhibited excellent inhibitory effects, and had similar effects on VSC production; however, the two agents had different anti-VSC kinetics. Briefly, 0.2% CHX had a sustained inhibitory effect, whereas Zn^{2+}had an immediate effect. In contrast, 0.2% CPC had only a mild inhibitory effect on VSC production. These ingredients are found in commercial mouthwashes, often in combination. Roldán et al. [27] compared five commercial mouthwashes in a randomized, double-blind, crossover trial: 0.12% CHX alone, 0.12% CHX plus 5% alcohol, 0.12% CHX plus 0.05% CPC, 0.12% CHX plus sodium fluoride, and a combination of 0.05% CHX, 0.05% CPC, and 0.14% Zn^{2+}. In this study, the combination of 0.12% CHX plus 0.05% CPC resulted in the greatest reduction in oral bacterial numbers. In contrast, the combination of 0.05% CHX, 0.05% CPC and 0.14% Zn^{2+}provided the most immediate reduction in VSC levels. Zn^{2+}can be effective in reducing the activity of VSCs directly, in addition to its antimicrobial effect [28]. It has been reported that a combination of $Zn^{2+}$and CHX or CPC inhibited VSC formation synergistically [29]. ClO_2 and chlorite anion (ClO_2^-) also oxidize VSCs directly into non-malodorous products, which consumes the amino acids that act as precursors to VSCs [30, 31]. A randomized double-blind crossover placebo-controlled clinical trial found that mouth rinsing with ClO_2 effectively reduced morning malodor for 4 h in healthy volunteers [32]. Triclosan is a broad-spectrum antibacterial agent that blocks lipid synthesis in susceptible bacteria [33]. A double-blind, crossover, randomized study comparing the VSC-reducing effects of mouthwashes on morning bad breath in healthy subjects reported that VSC formation was inhibited by, in descending order, mouthwashes containing 0.12% CHX gluconate, 0.03% triclosan, essential oils, and 0.05% CPC [34].

However, there are concerns regarding the potential side effects of these chemical agents. The use of 0.2% CHX results in an unpleasant bitter taste, perturbs taste, causes desquamative lesions and soreness of the oral mucosa, and yellow/brown staining of the teeth and dorsum of the tongue [35]. Hypersensitivity to CHX is rare, but several immediate-type allergies such as contact urticarial, occupational asthma, and anaphylactic shock have been reported [36, 37]. In Japan, based on these reports, the concentration of CHX used near a wound is limited to 0.05%, which is lower than its effective concentration. Recently, the possibility that triclosan is hazardous to human health has been suggested. Several studies reported that triclosan might contribute to bacterial resistance to antibiotics, or interfere with endocrine functions in rats [38, 39]. The US Food and Drug Administration (FDA) named triclosan in the National Toxicology Program (NTP) for toxicological evaluation.

3. Naturally derived compounds (Table 3)

3.1. Natural botanical extracts

Due to the increase in health consciousness, many flavors and natural botanical extracts have been added to foods and medicine to reduce oral malodor. In addition, the effects of natural botanical extracts on oral malodor have been evaluated in randomized controlled trials.

Study	Conditions for the assessment (the period that avoided oral activities, mouth cleaning, etc.)	Study population (Age)	Study design	Follow-up time	Active ingredient	Study group	Sample size	Pretreatment	Vehicle	Frequency (washout period)	Malodor assessment	Results
Natural botanical extracts												
Tanaka et al [41].	4:30–6:30 pm (at least 4 h)	Volunteers with gingivitis or mild periodontitis (20–50 years)	Double-blind, randomized, placebo-controlled parallel trial	14 weeks	Eucalyptus extract	High-concentration (0.6%), low-concentration (0.4%), and placebo	32, 32, and 33, respectively	Full-mouth supragingival scaling	Chewing gum	Two tablets for 5 min, five times daily for 12 weeks	OLT score, VSCs by GC	The OLT score decreased significantly at 4, 8, 12 and 14 weeks in the 0.4%- and 0.6%-eucalyptus extract groups but not in the placebo group. The group-time interactions revealed significant reductions in the OLT score and VSCs in both experimental groups compared with the placebo group.
Rassameemasmaung et al [47].	7:00–8:30 am (at least 2 h)	Gingivitis patients (18–55 years)	Double-blind, placebo-controlled parallel trial	4 weeks	Green tea extract	Green tea and placebo	Both $n = 30$	None	Mouthwash	Twice daily for 4 weeks	VSCs by Halimeter	The VSC levels decreased significantly at 30 min, 3 h, and day 28 in the green tea group. On day 28 there was a significant difference between the green tea and placebo group.
Rassameemasmaung et al [49].	8:00 am (at least 2 h)	Gingivitis patients (17–37 years)	Double-blind, randomized, placebo-controlled parallel trial	8 weeks	The pericarp extract of *Garcinia mangostana* L	Garcinia and placebo	Both $n = 30$	1) None 2) Scaling	Mouthwash	Twice daily for 2 weeks (4 weeks)	VSCs by Halimeter	1) The VSC levels decreased significantly in the Garcinia group compared with baseline and the placebo group. 2) The VSC levels in the Garcinia group was reduced significantly compared with the placebo group, but not with baseline.
Iha et al [52].	At the same time of day (at least 5 h)	Patients with oral malodor (33–71 years)	Randomized, open-label, parallel trial	4 weeks	Hinokitiol	Hinokitiol and 0.01% CPC	Both $n = 9$	None	Gel	Three times daily, for 4 weeks	OLT score, H_2S and CH_3SH levels using GC	The OLT score, and the levels of H_2S and CH_3SH were reduced significantly in the hinokitiol group, whereas the OLT score was improved significantly in the 0.01% CPC group.
Nohno et al [54].	Morning (at least 4 h)	Male volunteers (24–54 years)	Double-blind, randomized, placebo-controlled crossover trial	4 weeks	Actidinine	Actidinine and placebo	Both $n = 14$	None	Tablet	Three times daily, for a week (2 weeks)	VSCs by Oral Chroma	The VSC levels were reduced significantly in both the test and placebo groups after just taking a tablet. The VSC level was reduced significantly in the test group, but not in the placebo group, after use for 1 week.

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Study	Conditions for the assessment (the period that avoided oral activities, mouth cleaning, etc.)	Study population (Age)	Study design	Follow-up time	Active ingredient	Study group	Sample size	Pretreatment	Vehicle	Frequency (washout period)	Malodor assessment	Results
Salivary components												
Shin et al [57].	Morning (from the midnight before)	Volunteers with oral malodor (26–54 years)	Double-blind, randomized, placebo-controlled crossover trial	1 week	Lactoferrin and Lactoperoxidase	Lactoferrin and Lactoperoxidase, and placebo	Both $n = 15$	None	Tablet	Twice at a 1-h interval in the morning (1 week)	H_2S, CH_3SH, and total VSCs by GC	The CH_3SH level was significantly lower in the test group compared with the placebo group 10 min after the first ingestion. The median concentration of CH_3SH in the test group was below the olfactory threshold from 10 min until 2 h, whereas the level in the placebo group remained above the threshold during the experimental period.
Probiotic bacteria												
Kang et al [58].	7:00–8:00 am (from the evening before)	Student volunteers (20–30 years)	Open label crossover trial	1 day	*Weissella cibaria* CMU	*W. cibaria* CMU, *Lactobacillus casei*, *Weissella confuse*, and distilled water	46, 10, 10, and 46, respectively	None	Solution	15 mL for 2 min, twice daily	H_2S and CH_3SH using Oral Chroma	Rinsing of the mouth with solutions containing *W. cibaria* CMU twice a day significantly reduced H_2S and CH_3SH the next morning; *L. casei*, *W. confuse*, and distilled water had no effect.
Burton et al [63].	Morning (from awakening)	Subjects with oral malodor (18–69 years)	Open label parallel trial	1 week	*Sterptococcus salivarius* K12	*S. salivarius* K12 and placebo	13 and 10	Mechanical and chemical oral cleansing treatment	Lozenge	Day 1: at 2-h intervals over 8 h. Afterwards: twice daily for a week	VSCs by Halimeter	The VSC levels 1 week after treatment initiation was reduced significantly in the test group compared with the placebo group.
Keller et al [70].	Morning (from the evening before)	Young adult volunteers (19–25 years)	Double-blind, randomized, placebo-controlled crossover trial	7 weeks	*Lactobacillus reuteri* DSM 17938 and ATCC PTA 5289	*L. reuteri* and placebo	Both $n = 25$	None	Chewing gum	Twice daily for 2 weeks (3 weeks)	OLT, VSCs by Halimeter	The OLT score was significantly lower in the probiotic group compared with the placebo group. The VSC levels were not significantly different between groups.
Suzuki et al [76].	At the same time of day (at least 5 h)	Patients with oral malodor (22–67 years)	Double-blind, randomized crossover trial	6 weeks	*Lactobacillus salivarius* WB21	*L. salivarius* WB21 and placabo	Both $n = 23$	None	Tablet	Three daily for 2 weeks (2 weeks)	OLT, VSCs by GC	The OLT score was reduced significantly in both the probiotic and placebo periods. VSC levels were reduced significantly in the probiotic period but not in the placebo period.

OLT, organoleptic test; VSCs, volatile sulfur compounds; GC, gas chromatography; H_2S, hydrogen sulfide; CH_3SH, methyl mercaptan.

Table 3. Clinical trials to evaluate the effects of naturally derived compounds on reducing oral malodor.

Eucalyptus extract is one of the four active ingredients of Listerine® mouthwash (Pfizer Inc., Morris Plains, NJ, USA), which was created in 1879 and was formulated originally as a surgical antiseptic. It has antibacterial activity against several periodontopathic bacteria including *P. gingivalis* and *P. intermedia*, which produce VSCs [40]. The effect on oral malodor of chewing gum containing eucalyptus extract was evaluated in a double-blind randomized trial over a 12-week period [41]. Relative to baseline, organoleptic test (OLT) scores decreased significantly at 4, 8, 12, and 14 weeks in the 0.4%-and 0.6%-eucalyptus extract groups, but not in the placebo group. In addition, the group-time interactions revealed significant reductions in OLT scores, VSC levels, and tongue-coating scores in both eucalyptus concentration groups compared with the placebo group.

The catechins present in green tea have *in vitro* bactericidal activity against the odor-producing periodontal bacteria *P. gingivalis* and *Prevotella* spp. [42], inhibit the adherence of *P. gingivalis* to oral epithelial cells [43], and reduce periodontal breakdown by inhibiting the collagenase and cysteine proteinase activity of *P. gingivalis* [44, 45]. It was reported that green tea powder reduced VSC concentrations in mouth air immediately after administration [46]. A double-blind placebo-controlled clinical trial found that rinsing the mouth with green tea containing mouthwash twice per day significantly reduced VSC levels at 30 min, 3 h, and day 28, compared with baseline [47]. There was a significant difference between the green tea group and the placebo group at day 28 [47].

Pericarp extracts of *Garcinia mangostana*, which is commonly known as the mangosteen tree, exert antimicrobial activity against the oral bacteria *Streptococcus mutans* and *P. gingivalis*, and exhibit anti-inflammatory effects [48]. The use of mouthwash containing pericarp extracts of *G. mangostana* twice daily for 2 weeks reduced VSC levels significantly compared with baseline and the placebo group [49]. Furthermore, rinsing with mouthwash containing *G. mangostana* L for 2 weeks after scaling and polishing reduced VSC level significantly compared with placebo, whereas there was no significant difference between baseline and day 15 [49].

Hinokitiol (β-thujaplicin), a component of essential oils isolated from Cupressaceae, shows antibacterial activity against various bacteria, including periodontopathic bacteria and fungi [50, 51], and has been used as a therapeutic agent against periodontal disease and oral *Candida* infections. An open-label, randomized, controlled trial was performed in patients with genuine halitosis to evaluate the effects of mouth cleaning using hinokitiol-containing gels on oral malodor [52]. Mouth cleaning, including the teeth, gingiva, and tongue, was performed three times per day for 4 weeks. Organoleptic test (OLT) scores, levels of H_2S and CH_3SH, the frequency of bleeding on probing, mean probing pocket depths, and plaque indices were improved significantly in the group treated using the hinokitiol-containing gel. In contrast, only OLT scores improved significantly in the control group treated using 0.01% CPC-containing control gel.

Actidinine is a cysteine protease derived from the kiwi fruit. Tongue coating is understood to be an important factor in oral malodor and is composed of proteins [22, 53]. The effect of a tablet containing actidinine on oral malodor was evaluated in a double-blind, randomized crossover trial [54]. The subjects sucked the tablets three times per day for 1 week. VSC levels and tongue-coating ratios decreased significantly on the first day in both the test and placebo

groups immediately after taking a tablet. VSC levels were significantly lower after 7 days only in the test group. There was no significant reduction in tongue-coating ratios in either group after 7 days of use.

3.2. Salivary components

Saliva contains a variety of antimicrobial proteins including lactoferrin, peroxidase, lysozyme, and secretory immunoglobulin A. Lactoferrin is an iron-binding glycoprotein that chelates two ferric ions per molecule, and decreases bacterial growth, biofilm development, iron overload, reaction oxygen formation, and inflammatory processes [55]. Salivary peroxidase, in the presence of H_2O_2 and SCN-, can reversibly inhibit bacterial enzyme and transport systems by oxidizing the sulfhydryl groups of proteins [56]. A reduction in salivary flow might inhibit antimicrobial defense systems in saliva. A relationship between low salivary flow and the generation of H_2S and CH_3SH in mouth air has been reported previously [8].

The effect of a tablet containing lactoferrin and lactoperoxidase purified from bovine milk on oral malodor was evaluated in a randomized, double-blind, crossover, placebo-controlled clinical trial [57]. According to that study, CH_3SH levels were significantly lower in the test group compared with the placebo group 10 min after taking a tablet. The median CH_3SH concentration in the test group was below the olfactory threshold between 10 min and 2 h, whereas the level in the placebo group was above the threshold throughout the experimental period.

3.3. Probiotic bacteria

The use of probiotics as preventative and therapeutic products for oral healthcare is a novel antimicrobial approach that has been proposed as an alternative to chemotherapeutics. Probiotics are defined as "live microorganisms that confer a health benefit on the host when administered in adequate amounts" by the World Health Organization and the Food and Agriculture Organization of the United States (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf). Probiotics have been used traditionally to treat diseases related to the gastrointestinal tract. Recently, the use of such probiotics to improve oral health has attracted increasing attention, although this field is still in its infancy. Nevertheless, there are several reports related to the use of probiotics to ameliorate oral malodor.

Kang et al. isolated three peroxide-generating lactobacilli, identified as *W. cibaria*, from the saliva of kindergarten children aged 4–7 years who had little supragingival plaque and no oral disease, including dental caries [58]. These isolates co-aggregated with *F. nucleatum*, inhibited VSC production by *F. nucleatum*, and prevented proliferation by *F. nucleatum in vitro*. Subsequently, the effect of *W. cibaria* CMU on morning odor was evaluated in a clinical trial of healthy volunteers. Rinsing the mouth using solutions containing *W. cibaria* CMU twice per day reduced production of H_2S and CH_3SH the next morning significantly. Conversely, use of solutions containing distilled water, *Lactobacillus casei,* and *Weissella confusa* had no effect.

Streptococcus salivarius K12 has been used to prevent the pharyngitis and tonsillitis induced by *Streptococcus pyogenes*. *S. salivarius* was selected as an oral probiotic because it is an early

colonizer of oral surfaces and is the predominant member of tongue microbiota numerically in 'healthy' individuals [19, 59]. *S. salivarius* K12 produces two bacteriocins: salivaricin A and salivaricin B [60, 61]. It exerts inhibitory activities against oral malodor-related oral bacteria, such as *Atopobium parvulum*, *Eubacterium sulci*, and *S. moorei*, to varying extents [62]. According to an additional *in vitro* study, inhibitory effects were observed against *Streptococcus anginosus*, *Eubacterium saburreum*, and *Peptostreptococcus micros*, but not *P. gingivalis* and *P. intermedia* [63]. This report described the results of a preliminary clinical trial that administered lozenges containing either *S. salivarius* K12 or placebo. The subjects undertook a 3-day regimen of CHX mouth rinsing followed by the use of lozenges at specific intervals. The VSC levels 1 week after the initiation of treatment were reduced significantly in the *S. salivarius* K12 group compared with the placebo group. The salivary bacterial composition was examined using PCR-denaturing gradient gel electrophoresis, and data revealed that it changed in most subjects following K12 treatment, albeit to differing extents.

Lactobacillus reuteri is a member of the indigenous oral microbiota in humans, and it exerts antibacterial properties by converting glycerol into reuterin, a broad-spectrum antimicrobial substance [64]. Products that contain *L. reuteri* have been marketed for the prevention and treatment of gingivitis and periodontal disease [65-67]. However, data are conflicting regarding the potential of *L. reuteri* for caries management, as some studies reported useful effects whereas other did not [68, 69]. The effect of chewing gum containing two strains of probiotic lactobacilli (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289) on oral malodor was evaluated in a randomized double-blinded placebo-controlled crossover trial [70]. The study populations were healthy volunteers, and the study design included two intervention periods of 2 weeks with a 3-week washout period. The organoleptic scores were significantly lower in the probiotic group compared with the placebo group. However, there were no differences in VSC levels between the two groups, either before or after rinsing with L-cysteine. The researchers hypothesized that the probiotic gum might have affected bacteria that produce malodorous compounds other than VSCs.

Lactobacillus salivarius WB21 is an acid-tolerant lactobacillus derived from *L. salivarius* WB1004 [71], and is a potentially effective probiotic against *Helicobacter pylori*. Oral consumption of tablets containing *L. salivarius* WB21 was reported to improve periodontal conditions in healthy volunteer smokers and reduce the numbers of the periodontopathic bacterium *T. forsythia* in subgingival plaque [72, 73]. A double-blind, randomized, placebo-controlled clinical trial using oils containing *L. salivarius* WB21 in patients with periodontal disease reported reduced bleeding on probing compared with the placebo group after 2 weeks [74]. We performed an open-label pilot study previously to evaluate whether oral administration of a tablet containing *L. salivarius* WB21 altered oral malodor or clinical conditions in patients complaining of oral malodor [75]. The organoleptic scores and concentrations of H_2S and CH_3SH were reduced in patients without periodontitis after 2 weeks of treatment, and the organoleptic scores and bleeding on probing were decreased in patients with periodontitis after 4 weeks. Subsequently, we performed a 14-day, double-blind, randomized, placebo-controlled crossover trial using tablets containing *L. salivarius* WB21 or placebo taken orally by patients with oral malodor [76]. The organoleptic scores were decreased significantly in

both the probiotic and placebo periods compared with the baseline scores, and there was no difference between periods. Compared with the values at baseline, the concentrations of total VSCs decreased significantly in the probiotic period but not in the placebo period, and significant differences were observed between the two periods. In addition, the mean probing pocket depth decreased significantly in the probiotic period compared with the placebo period. Quantitative analysis of the bacteria in saliva found significantly lower levels of ubiquitous bacteria and *F. nucleatum* during the probiotic period.

4. Conclusions

Chemical agents have been used widely to prevent and treat oral malodor. However, long-term use of some antiseptic agents such as CHX might result in complications such as staining of teeth and the development of microbial resistance. In addition, recent studies have raised concern regarding the potentially harmful effects of triclosan on the human body. These phenomena and consumers' increasing health consciousness have led to the development of alternative antimicrobial approaches, including herbs, natural botanical extracts, salivary components, and probiotics. Diverse natural products have been marketed as effective for preventing and treating oral malodor, and an increasingly diverse range of strategies for oral malodor is available. However, few studies have demonstrated effectiveness of new products against oral malodor clinically. Furthermore, most studies evaluated the short-term effects of products on oral malodor, either immediately or only a few weeks after taking the products. However, the products used for preventing and treating oral malodor, including mouthwash, toothpaste, tablets, and lozenges, are generally used for the long term. Therefore, the long-term effects of agents on oral malodor, as well as their safety and side effects, should be evaluated in randomized controlled trials.

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