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The Effect of *Streptococcus salivarius* K12 on Halitosis: a Double-Blind, Randomized, Placebo-Controlled Trial

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Abstract
This study was to evaluate the effect of *Streptococcus salivarius* K12 on tongue coating–associated halitosis. Twenty-eight subjects having tongue coating–associated halitosis were randomly divided into either a test or control group. For each of the 30 days, the test subjects sucked *S. salivarius* K12 tablet while the control subjects sucked placebo tablets. All the subjects did not take physical (tongue scraping) and chemical (antiseptic mouth-rinse) oral cavity pretreatment prior to use of the tablets. At baseline, and on the 1st, 7th, and 14th day after completing the course of tablets, the subjects were assessed for their organoleptic test (OLT) scores, volatile sulfur compound (VSC) levels, and tongue coating scores (TCS). During the course, all subjects kept their routine oral care habits without scraping their tongue coating. Plaque index, probing depth, and bleeding index were recorded at baseline and at the completion of the trial. On the 1st day following the end of tablet use, the OLT scores and VSC levels had significantly decreased in the test group when compared with the baseline values ($P = 0.001$ and $P = 0.012$). The TCS in the test group were also significantly decreased ($P = 0.05$). At days 7 and 14, the OLT scores in the test group were still significantly lower than the baseline levels ($P = 0.006$ and $P = 0.039$ respectively). However, there were no statistical differences with OLT, VSC, and TCS between the test group and the placebo group by analysis of multi-level regression model. The use of *S. salivarius* K12 did not have significant effect on halitosis with tongue coating cause when the tongue coating was not physically or chemically pre-treated, which implies removing tongue coating is required before *Streptococcus salivarius* K12 use.

Keywords Halitosis · Tongue coating · Probiotics · *Streptococcus salivarius* K12

Introduction

Halitosis can be clinically classified as genuine halitosis, pseudo-halitosis, and halitophobia [1]. Pseudo-halitosis refers to the situation where the patient does not have an actual breath odor problem (nothing can be detected by smell or scientific testing), but they are still certain that they do have bad breath [1, 2]. Halitophobia refers to the situation where a patient's perception of a breath problem continues to exist despite the successful treatment of their genuine halitosis condition, or in the case of pseudo-halitosis, after receiving counseling [1]. Genuine halitosis is used when the malodor really exists and can be diagnosed organoleptically or by measurement of the responsible compounds. It includes oral halitosis and extra-oral halitosis [3], with 80–90% of genuine halitosis being from the oral cavity [3, 4]. Tongue coating and periodontitis are considered the main etiology of oral halitosis. It was reported that tongue coating was the principal cause of halitosis among patients who complained of bad breath in a halitosis clinic (43.4% tongue coating only, 18.2% tongue coating combined with gingivitis/periodontitis) [5]. Undoubtedly, the tongue is a major site of halitosis production, with other factors contributing less substantially to the overall problem [5, 6]. It is due to the large area of the tongue dorsum, especially the posterior region that retains large numbers of desquamated cells, leukocytes, salivary constituents, and microorganisms.

Subjects having halitosis have more diverse microbial populations of the tongue dorsum than those found in healthy subjects [7–9]. Halitosis results from microbial degradation of organic substrates present on the tongue dorsum, in the

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saliva and periodontal pocket, retained debris, and metabolites of the oral microorganisms themselves. The most common compounds associated with halitosis are volatile sulfur compounds (VSC), such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3) [10].

Halitosis can be treated either by reducing the numbers of oral bacteria or by modifying the composition of the oral microbiota. At present, the principal treatment for halitosis originating from the tongue coating is mechanical cleaning. Another commonly-applied adjunctive approach is chemical therapy using various antibacterial agents, metal ions (Zn^{2+}, Cu^{2+}, Fe^{2+}, etc.), essential oils, or oxidants [11]. However daily tongue cleaning procedures are very uncomfortable, and chemical therapy can have side effects, such as dysbacteriosis, staining, and transient altered taste [12]. It was reported that mechanical self-cleaning of tongue coating, though may relieve malodor to a certain extent, actually had less effect on tongue coating–derived halitosis for a long duration if without intervention by dentist [13]. Thus, more effective, convenient, and comfortable methods are still needed to be developed to aid resolution of malodor originating from tongue coating.

Probiotic treatment of halitosis has been introduced recently. Henker et al. [14] reported firstly that a girl with malodor of intestinal origin was successfully treated with a non-pathogenic strain of *Escherichia coli*. Subsequently, there have been several reports of possible beneficial role in the treatment of halitosis using probiotic strains *Streptococcus salivarius* K12 [15–18], *Weissella cibaria* [19], and *Lactobacillus salivarius* WB21 [20–23]. *S. salivarius* does not cause dental caries or other oral infections. It is a predominant species on the dorsum of the tongue in healthy subjects [7–9]. Moreover, it does not produce VSCs contributing to halitosis while having an important role in maintaining the ecological balance of the host's oral microflora [15]. *S. salivarius* K12 was originally isolated from the oral cavity of a healthy child. The daily ingestion of *S. salivarius* K12 over a 28-day period does not adversely affect the human host according to blood and urine test in healthy volunteers [24]. However, only two trials have evaluated the effect of *S. salivarius* K12 on halitosis in vivo [15, 25] up to date. In adult subjects with halitosis, VSC in test group who were sucking lozenge of *S. salivarius* K12 reduced significantly at 1 week than that in control group who were sucking lozenge but without bacteria [15]. However, only a few subjects were observed for their VSC changes in a longer time. Their VSC changed inconsistently and returned to the levels before treatment or maintained in the lower level [15]. While in children, malodor reduction of organoleptic test (OLT) persisted to 3 months after cessation of 2-week sucking of *S. salivarius* K12 [25]. Preventing reestablishment of undesirable bacteria and thereby preventing the reoccurrence of oral malodor by probiotics are supposed to be the hypothesis of the above two studies. For chlorhexidine rinse and scraping of tongue coating, both were conducted before probiotic use [15, 25]. In the studies, the cause of the subjects' halitosis was not recorded, and the tongue coating index was not measured either [15, 25]. Based on the ability of probiotics to compete with pathogenic microorganisms for adhesion sites and to antagonize these pathogens [16, 17], we hypothesize that malodor with tongue coating cause may be alleviated by *S. salivarius* K12. However, how far the benefit may be achieved without pretreatment of tongue coating still needs to be proved.

Thus, the present study was designed to be a double-blind, randomized, placebo-controlled evaluation of the treatment effect of *S. salivarius* K12 on halitosis of tongue coating origins.

Materials and Methods

Subjects

Patients in the Department of Periodontology, Peking University School and Hospital of Stomatology, who had tongue coating–derived halitosis, were included. The inclusion criteria were (1) organoleptic test (OLT) score ≥ 2; (2) oral cavity VSC ≥ 150 ppb by a halimeter (Interscan Corp., Chatsworth, CA, USA); (3) score of area of tongue coating ≥ 2 and score of thickness of tongue coating ≥ 2; (4) absence of tongue cleaning habit; (5) probing depth (PD) ≤ 4 mm, attachment loss(AL) ≤ 2, bleeding index (BI) ≤ 2, percentage of sites with bleeding on probing (BOP%) ≤ 25%; (6) absence of dental caries, apical periodontitis, poor restorations, and impacted wisdom teeth; (7) no smoking; (8) without systemic illness; (9) no use of antibiotics for the past 3 months.

A total of 33 patients complaining of halitosis from May 2014 to March 2016 were initially enrolled in the study. Four patients were of periodontal health. The other 29 patients were with gingivitis or mild periodontitis originally and received successful periodontal treatment before enrolling in this study. When we re-evaluated, they matched the inclusion criteria. Five were finally excluded: one used antibiotic and four did not recall on time. Thus, 28 patients (8 males, 20 female) finally completed the study. The power is 0.88 ($n = \frac{2\left(\mu_\alpha+\mu_\beta\right)^2 p(1-p)}{\delta^2}$, $\alpha = 0.05$, $p = (p_1 + p_2)/2$, $\delta = p_1 - p_2$, $p_1 = 0.85$, $p_2 = 0.3$, $n = 13$). Informed consent was obtained from all patients. The study protocol and the procedure used for obtaining informed consent were approved by the Ethics Committee of the Peking University School and Hospital of Stomatology (PKUSSIRB-201412026).

Procedures

This study was conducted as a randomized, controlled, double-blinded trial. The participants were assigned by simple randomization according to the order of their registration either to the test or control group. Random numbers were derived from the table of random numbers by Doctor Chen who was also responsible for allocating the tablets. The test and placebo tablets were contained within identical packaging and were distributed according to the random serial number, and all the tablets were manufactured by and directly provided for use by BLIS Technologies Ltd. After finishing the study, we knew the grouping. The test group subjects sucked lozenges containing 1×10^9 colony forming units (CFU) of *S. salivarius* K12 (BLIS Technologies Ltd., Dunedin, New Zealand). Subjects in the control group sucked placebo lozenges (BLIS Technologies Ltd., Dunedin, New Zealand) that had not been supplemented with *S. salivarius* K12. The patients were instructed to suck a tablet twice daily following tooth brushing in the morning and evening for 30 days. After using the tablets, the patients should not eat or drink anything and not conduct any oral hygiene activities for at least 1 h. They were also instructed not to change their usual oral hygiene regimens and not to take any other probiotic products throughout the study period. The examiner and patients had no knowledge of the group assignments.

The parameters of examination included OLT score and VSC level by a halimeter, area of tongue coating (Ta), thickness of tongue coating (Tt), plaque index (PLI), PD, BI, and BOP%. Before the trial, inter-calibration with a standard examiner and intra-calibration of OLT were performed. The Kappa was 0.857 and 0.870, respectively.

At baseline, before use of the tablets, each subject was examined by the examiner. The parameters were recorded in the following order: OLT and VSC firstly, then tongue coating, and finally periodontal indexes (PLI, PD, BI, BOP%). On the1st, 7th, and 14th day following the 30-day course of tablet-taking, OLT, VSC, Ta, Tt, and PLI were reexamined. On the 14th day after stopping taking the tablets, other parameters including PD, BI, and BOP% were recorded. The trial flowchart is shown in Fig. 1.

Clinical Examinations

Malodor-Related Parameter Assessment

Malodor assessment was conducted by a single dentist. The OLT score and the total VSC concentration were evaluated as the major outcomes. Assessments were conducted in the morning between 8:00 to 10:00 am. In the 24 h before odor testing, the patients were not allowed to eat onions, garlic, leeks, and other odorous food, or to drink alcohol. In the 2 h before testing, the patients also were not allowed to eat, to drink beverages, to chew gums, or to brush their teeth.

The OLT score included a scale of 0 to 5 [26]. The patients closed their mouth for 1 min, and then exhaled slowly from their mouth into the clinician's face from a 10-cm distance. The score was evaluated as follows: 0 = none, 1 = barely noticeable, 2 = slight but clearly noticeable, 3 = moderate, 4 = strong offensive, and 5 = extremely foul. The reduction of OLT score was considered to be effective.

The VSC concentration was tested by a halimeter according to the manual. Values of VSC were recorded based on the average of three readings.

Area and thickness of tongue coating were determined by inspection. Ta was determined on a scale of 0–3 (0, none visible; 1, less than one-third of tongue dorsum surface covered; 2, less than two-thirds; and 3, more than two-thirds). Tt was recorded as a score of 0–2 (0, no tongue coating; 1, thin tongue coating: tongue papillae visible; and 2, thick tongue coating: tongue papillae invisible). Then, the tongue coating score (TCS) was obtained by multiplying Ta by Tt [27].

Evaluation of Periodontal Status

A periodontal examination was performed, and the periodontal parameters included PLI (Silness and Löe, 1964), PD, and BI (Mazza, 1981). The PLI was evaluated for 6 sites (mesial-buccal, buccal, distal-buccal, mesial-lingual, lingual, distal-lingual) of the Ramfjörd index teeth (16, 11, 24, 36, 41, 44) per patient. The PD and BI were evaluated on all teeth, 6 sites per tooth. Percentage of sites with bleeding on probing was calculated at baseline.

Statistical Analysis

Clinical data was entered and analyzed using SPSS 19.0. Demographic characteristics and periodontal data (PD, BI, and PLI) were evaluated by *t* test. The OLT scores, value of VSC, and tongue coating scores of the two groups were analyzed by the Mann-Whitney *U* test. The data was also analyzed by the Mann-Whitney *U* test in each group when compared with the baseline values. Multi-level linear regression models were used to determine the effects of *S. salivarius* K12 on clinical outcomes at multiple time points. If the $p < 0.05$, the decrease was statistically significant.

Results

Twenty-eight subjects completed the study and none reported any adverse events.

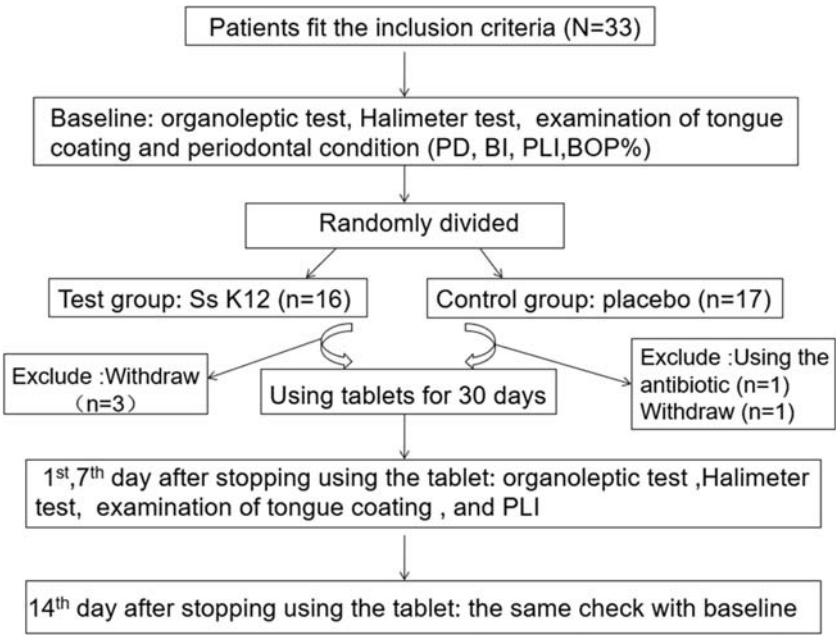

Fig. 1 Flowchart of the trial. A double-blind, randomized, placebo-controlled trial was conducted in patients with halitosis of tongue coating cause (Ss K12: *Streptococcus salivarius* K12)

Baseline characteristics of the participants

The test group included 13 patients with 6 males and 7 females, while the control group included 15 patients with 2 males and 13 females. The gender constitution was not significantly different between the two groups. The average age of all patients was 30.04 ± 7.00 years with a range of 23–44 years. The average age of the test group was 32.54 ± 6.79 years and of the control group 27.87 ± 6.64 years. No significant difference was found between the two groups.

At baseline, the subjects in both groups showed mild to moderate malodor when evaluated by their OLT scores, VSC values, and TCS. These parameters did not show any significant difference between the two groups (Table 1, 2, and 3).

No significant differences of periodontal parameters were observed between the two groups at baseline. In the two groups, the average PD was less than 3 mm, the average BI was less than 2, and the average PLI was less than 1, which showed that their periodontal condition was relatively healthy and that the subjects had good oral hygiene (Table 5 and 6).

Changes of Halitosis Parameters in each Group

OLT score

On the 1st day after stopping tablet usage (day 1), both groups showed a significantly decreased OLT score when compared with the baseline values ($p < 0.05$) (Table 1).

In order to detect any persisting effect of taking the *S. salivarius* K12 tablets, the OLT scores on the 7th (day 7) and 14th (day 14) day after stopping using the tablets were observed. It was found that although the OLT scores on days 7 and 14 had increased in both groups by comparison with the day 1 scores, the values remained significantly less than at baseline for the test group patients (Table 1).

VSC Level

At day 1, the VSC levels had decreased in both groups when compared with the baseline scores. In the test group the decrease was statistically significant ($p < 0.05$) (Table 2).

Table 1 Organoleptic test score (OLT) before and after use of the tablets

		Baseline	After stopping the use of tablets		
	n		Day 1	Day 7	Day 14
Test group	13	2.85 ± 0.69	1.38 ± 0.96	1.77 ± 0.93	2.15 ± 0.69
P value			0.001*	0.006*	0.039*
Control group	15	2.47 ± 0.64	1.40 ± 1.55	1.73 ± 1.28	1.80 ± 1.21
P value			0.049*	0.137	0.137
P value between groups		0.363	0.964	0.856	0.555

*$p < 0.05$ compared with the baseline

Table 2 The volatile sulfur compound level (ppb) before and after use of the tablets

		Baseline	After stopping the use of tablets		
	n		Day 1	Day 7	Day 14
Test group	13	335.15 ± 164.48	183.46 ± 95.85	241.54 ± 191.70	242.08 ± 138.67
P value			0.012*	0.081	0.072
Control group	15	295.2 ± 162.19	210.2 ± 160.75	203.80 ± 171.31	232.0 ± 145.79
P value			0.126	0.023*	0.202
P value between groups		0.217	0.821	0.821	0.467

*$p < 0.05$ compared with the baseline

Indicative of a persisting beneficial effect, we also found that although the VSC levels on day 7 and day 14 had increased from their day 1 levels, these were still less than the baseline levels. In the control group the decrease was statistically significant ($p < 0.05$) compared with baseline at day 7 (Table 2) but not statistically significant at day 14.

Changes in Tongue Coating Score

On the 1st day after stopping using the tablets, the TCS had significantly decreased in the test group when compared with the baseline scores ($P = 0.05$). The scores also decreased in the control group but without statistical significance. At day 7, the TCS in the test group still had a trend of decrease ($P = 0.064$). But there was no statistically significant difference between the test and control groups (Table 3).

Effect of *S. salivarius* K12 on Halitosis Reduction by Multi-Level Linear Regression Analysis

Multi-level regressions showed no significant effect of the treatment with *S. salivarius* K12 on all halitosis-related outcomes including OLT, TCS, and VSC. Comparisons of different time points within the group and between groups also did not show any significant effects (Table 4).

Changes in Periodontal Parameters

Before and after treatment (baseline vs day 14), the average PD, BI, and PLI did not show any statistically significant differences between and within the two groups. These results indicate that the periodontal condition of the two groups were stable and remained similar during the trial (Table 5 and 6).

Discussion

This study is a double-blind, randomized, placebo-controlled trial to investigate the effect of taking *S. salivarius* K12 tablets on halitosis with tongue coating causation. Without removing the tongue coating prior to the study, no significant effect was found after the end of the 30-day course of sucking *S. salivarius* K12 tablets when compared with placebo-tablet sucking.

To our knowledge, this is the first study of the efficacy of *S. salivarius* K12 tablets in the treatment of halitosis that is attributable primarily to excessive tongue coating. Several previous studies have reported that probiotics can be used to treat halitosis [15, 16, 19–21, 28]. However, none reported the clinically established causation of malodor in the subjects. Also none have recorded the effects of *S. salivarius* K12 tablets on tongue coating scores [15, 16].

It has been reported that the bacterium *S. salivarius* K12 can be detected at the mucosal surface after use of the tablets [29]. Strain K12 is known to produce at least two lantibiotic bacteriocins (salivaricin A and salivaricin B). These bacteriocins have been shown in vitro to inhibit the growth of representative strains of some bacteria implicated in the generation of halitosis [15, 17]. Strain K12 can also boost salivaricin levels in the oral cavity by cross-stimulating salivaricin

Table 3 Tongue coating score (TCS) before and after use of the tablets

		Baseline	After stopping the use of tablets		
	n		Day 1	Day 7	Day 14
Test group	13	3.85 ± 1.57	2.77 ± 1.77	2.85 ± 1.52	3.00 ± 1.41
P value			0.05	0.064	0.113
Control group	15	4.2 ± 1.78	3.2 ± 1.52	3.47 ± 1.41	3.87 ± 1.64
P value			0.14	0.39	0.71
P value between groups		0.751	0.496	0.13	0.13

*$p < 0.05$ compared with the baseline

Table 4 Multi-level regressions for treatment effects of *S. salivarius* K12

	Volatile sulfur compound level		OLT		Tongue coating score	
	β	*P* value	β	*P* value	β	*P* value
Group (treatment)	19.0	0.731	0.19	0.621	− 0.26	0.717
Time	− 22.1	0.147	− 0.17	0.126	− 0.29	0.135
Group × time	− 2.5	0.903	− 0.003	0.986	− 0.23	0.383

S. salivarius K12 Streptococcus salivarius K12

production by pre-existing members of the indigenous oral microbiome. Thus, taking tablets containing strain K12 may be beneficial by suppressing the growth of some odiferous oral pathogens [15]. The microbial composition on the tongue dorsum of patients with malodor has been shown to be more complex than that found in people without halitosis [7–9]. *Solobacterium moorei* has been commonly detected in people having tongue coating–borne malodor [7–9]. Five tested *S. moorei* isolates were inhibited in vitro by *S. salivarius* K12 [17]. Therefore, the use of *S. salivarius* K12 tablets may selectively and beneficially modify the microbial population on the tongue dorsum by inhibiting some of the species of bacteria particularly implicated in halitosis production theoretically.

Even though no significant effect of *S. salivarius* K12 on all halitosis parameters was found when compared with placebo in the present trial, we found that the tongue coating score of the subjects showed a decrease trend after the use of *S. salivarius* K12, especially decreased significantly at the first day after treatment ending. While in the placebo group, such phenomena was not shown. This means that the use of *S. salivarius* K12 may more or less have a potential beneficial influence on the microbiota on the tongue dorsum. However, this effect was too weak. The main reason was considered to be no mechanical or chemical removal of tongue coating in our patients. In the present study, the tongue coating was not pre-treated and even not removed during the trial, though all subjects had extensive tongue coating. As the pre-established tongue coating was a kind of biofilm, it has capability of preventing inside of the coating from interference of probiotics. The weak effect of *S. salivarius* K12 may only come from the surface layer change of the coating or the ongoing formation of the biofilm on the top surface of the coating. Pre-existing microbial environment in the tongue was almost not disturbed if the tongue dorsum was not cleaned prior to sucking the *S. salivarius* K12 tablets. These may explain the findings that no significant difference of OLT and VSC was shown between the test group and the placebo group, though reduction seemed better in test group.

However, *S. salivarius* K12 lozenges on halitosis indeed showed a promising effect though the cause of malodor in children subjects was not accurately introduced in a recent study [25]. Jamali et al. [25] observed the best effect of OLT reduction in the *S. salivarius* K12 group after pretreatment by removal of tongue coating and CHX rinsing in a case control study. OLT improvement increased gradually in the following order, treatment by toothbrush + flossing, toothbrush + flossing + tongue scraping, toothbrush + flossing + tongue scraping + CHX, and then increased significantly by toothbrush + flossing + tongue scraping + CHX + probiotics. It indicated that probiotic therapy following oral disinfection could be an effective approach for 3-month control of oral malodor. Based on the principle of competitive exclusion, a dramatic reduction in oral microbial loads provides a unique opportunity for easy colonization with probiotic strains. Since oral malodor might be caused mainly by tongue coating in the young and by periodontal diseases together with tongue coating in the older in general population [25, 30], we guess the malodor cause in children subjects were mainly tongue coating and poor oral hygiene [25]., while the earlier study [15] reported malodor quickly recovers once treatment of stops by a few example cases, which was not consistent with the above

Table 5 Probing depth (PD) and bleeding index (BI) before and after use of the tablets

	PD		BI	
	Baseline	Day 14[a]	Baseline	Day 14[a]
Test group ($n = 13$)	2.48 ± 0.10	2.47 ± 0.07	1.07 ± 0.34	1.15 ± 0.34
P value		0.663		0.561
Control group ($n = 15$)	2.44 ± 0.11	2.44 ± 0.11	0.98 ± 0.32	1.06 ± 0.30
P value		0.472		0.973
P value(between groups)	0.271	0.447	0.482	0.485

[a] Day 14 after stopping use of the tablets

P value: compared with the baseline

Table 6 Plaque index (PLI) before and after use of the tablets

		Baseline	After stopping the use of tablets		
	n		Day 1	Day 7	Day 14
Test group	13	0.94 ± 0.14	0.86 ± 0.15	0.90 ± 0.22	0.85 ± 0.13
P value			0.632	0.171	0.107
Control group	15	0.79 ± 0.27	0.82 ± 0.31	0.82 ± 0.40	0.81 ± 0.30
P value			0.821	0.788	0.850
P value(between groups)		0.08	0.666	0.494	0.646

P value: compared with the baseline

trial. In this randomized, placebo-controlled trial, significant VSC reduction was found during but not after the course of *S. salivarius* K12 tablet administration [15]. Removal of tongue coating together with disinfection of oral cavity by CHX for 3 days before probiotics use and observing VSC change during but not after the trial, may partially explain the inconsistent findings with ours and Jamali et al.'s [15].Thus, the next step in the future research should be taken to prove the effect of *S. salivarius* K12 on halitosis combining with removing the tongue coating for subjects with tongue coating cause. Approach for solving long-persistent tongue coating halitosis by probiotics alone was not recommended in the present study. It actually confirms the requirement for physical/chemical microbiota disruption to optimize *S. salivarius* K12 efficacy.

The organoleptic test and halimeter were both used to monitor halitosis in this research. The differences in the assessment by the two methods were due to them measuring different parameters in the oral cavity. The Halimeter™ only detects volatile sulfur compounds (VSC), and the detectors are more sensitive to H_2S than to CH_3SH and CH_3SCH_3. However, at the same concentration of CH_3SH and H_2S, the odor power of CH_3SH is three times that of H_2S [31]. Moreover, a much wider range of odors (such as indor, amine, etc.) besides VSC is detectable by organoleptic assessment. Thus, the organoleptic assessment is now being considered the "gold standard," [32–34] though organoleptic data are related to the operator. In our experience, it is preferable to use two ways to evaluate malodor in order to obtain a more complete evaluation. Thus, OLT and VSC, both parameters were observed for the first time in the present trial.

It is worthy to note some results in the two groups by monofactor analysis respectively. In the test group, daily sucking of S. *salivarius* K12 tablets for 30 days led to a significant decrease in tongue coating, in OLT scores, and in VSC levels. The significant decrease of OLT score was maintained for 14 days. The control group showed a significant decline in OLT scores and VSC levels; the decline did not persist for 14 days beyond taking of the tablets. Yet, the decline was not as evident as in the S. *salivarius* K12–treated group. The reason for a reduction in the control group may relate to the placebo effect [35]. Stress has been identified as a halitosis-inducing factor, since in humans can increase VSC levels [36]. The patients in the control group may be soothed psychologically due to the placebo effect. Secondly, the sucking of tablets can stimulate the secretion of saliva. Dilution of the saliva flow and the increase of immunity defense substance against odor-causing organisms may lead to some decrease in odor. These findings seem that the use of *S. salivarius* K12 tablets has a potential benefit on reducing the severity of halitosis of tongue coating origins if proper strategy is made. And sucking *S. salivarius* K12 tablets daily for some time may possibly maintain persisting effect quite a time on removing malodor with tongue cause. Horz et al. [29] reported that *S. salivarius* K12 can be detected at the mucosa surface for as long as 3 weeks following its entry to the oral cavity. The amount of *S. salivarius* K12 decreased steadily from the 8th day after stopping use [29]. On the basis of the previous observations, we have concluded that the reducing malodor may be due to the prolonged survival of *S. salivarius* K12 and the selective suppression on malodor-related microbiol by competitive growth on the tongue surface. In other words, probiotics use as an adjunctive method to relieve tongue coating–related malodor and maintain the efficacy persistently has more availability for preventive aim than for therapeutic aim.

Actually, our previous study reported that among patients with genuine halitosis, only 11.2% had ever tried professional instruction or treatment for bad breath [37]. Among patients visiting our clinic for the first time, 88.6% reported never cleaning tongue [37]. Moreover, mechanical self-cleaning of tongue coating only had limited effect on reduction of tongue coating–caused halitosis if without professional intervention [13]. Under such situation of low perception on proper care for treating halitosis, the effect of *S. salivarius* K12 use on tongue coating–caused malodor without pretreatment is unknown yet. In fact, which strategy is better for eliminating halitosis with tongue coating cause is still to be probed in the future because of less current evidence. Cleaning tongue coating mechanically should be a necessity. Probiotics use following it may relieve the uncomfortable sense and avoid the recurrence dramatically, for tongue scraping daily may be no longer necessary, as a practice with short period instead.

Even so, how long does probiotics should be used as a routine practice or a period of practice? Oral disinfection by CHX for a period of time may be helpful before beginning of probiotics therapy, while how long should it be rinsed is still a question. The two published studies so far on halitosis therapy with *S. salivarius* K12 showed inconsistent result [15]. Difference of malodor cause may be one reason. Meanwhile period of CHX rinsing may be another explanation. Mouth rinsing should have enough efficacies on suppressing odorous pathogens and reducing bacteria loads in oral cavity, when following the tongue cleaning and other routine oral hygiene practice, as well as with the lowest adverse effect. Therefore, proper strategy for solving halitosis with tongue coating cause still has quite more questions to be answered. In future studies, it would be instructive to monitor the specific changes occurring within the oral microbiome including the precise oral distribution achieved by *S. salivarius* K12. And longitudinal, large sample, double-blind, randomized, placebo-controlled trial needs to be developed in halitosis subjects with a clear cause either.

In conclusion, this study prompted that the use of *S. salivarius* K12 alone did not have significant effect on halitosis with tongue coating cause when the tongue coating was not mechanically removed or chemically pre-treated. Use of *S. salivarius* K12 with the prior removal of tongue coating may be required to optimize the efficacy of this treatment.

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Compliance with Ethical Standards

Conflict of Interests The authors declare that they have no conflict of interests.

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