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The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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5 1 **Title page**
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7 2 **Title:** The efficacy of probiotics in the management of halitosis: A systematic review and meta-
8 3 analysis

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16 11 **Running title:** Probiotic treatment of halitosis
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22 12 **ABSTRACT**
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24 13 **Objectives** Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have
25 14 been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as
26 15 an alternative method to alleviate halitosis. The present study aimed to investigate the effect of
27 16 probiotics on halitosis.

28 17 **Design and methods** This is a meta-analysis study. A search was performed in indexed databases up
29 18 to February 2021. Randomized controlled trials were included that compared probiotics and placebo
30 19 concerning primary outcomes of organoleptic scores and volatile sulfur compounds levels. Data
31 20 extraction and quality assessment were conducted independently by two reviewers.

32 21 **Results** Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to
33 22 synthesize data. The data were sub-grouped and analyzed in the short term (≤ 4 weeks) and long term
34 23 (> 4 weeks) based on the follow-up time. Seven articles were included in this review. For primary
35 24 outcomes in the short term (≤ 4 weeks), organoleptic scores [SMD= -0.58; 95%CI (-0.87, -0.30),
36 25 $p < 0.0001$] and volatile sulfur compounds levels [SMD= -0.26; 95%CI (-0.51, -0.01), $p = 0.04$]
37 26 significantly decreased in the probiotics group compared with the placebo group. However, a
38 27 significant reduction was observed only in organoleptic scores [SMD= -0.45; 95%CI (-0.85, -0.04),
39 28 $p = 0.03$] in the long term (> 4 weeks). No significant differences were observed in secondary outcomes
40 29 (tongue coating scores and plaque index).

41 30 **Conclusions** According to the results of this meta-analysis, it seems that probiotics can be used to
42 31 relieve halitosis in the short term (≤ 4 weeks). The results of bias assessment and limited data might
43 32 reduce the reliability of the conclusions.

44 33 **Strengths and limitations of this study**

- 45 34 ▶ This study included larger RCTs involved in halitosis and probiotics.
46 35 ▶ The results were rationally analyzed from the follow-up time perspective.
47 36 ▶ The included studies had limited patients.
48 37 ▶ Some studies reported the outcomes with different forms, increasing the heterogeneity of the
49 38 results.
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39 INTRODUCTION

40 Halitosis, also known as “oral malodor,” is typically defined as an unpleasant odor emanating from
41 the oral cavity.¹ As a cause of patients’ referral to the dentist, halitosis is the third most common
42 disease, only ranking behind dental caries and periodontal disease.² According to an epidemiological
43 study, the prevalence of halitosis is approximately 27.5% in the Chinese population.³ People have a
44 higher demand for social interactions and attach more importance to their personal image in today’s
45 society. Halitosis has a significant impact on both patients’ daily work and social activities and may
46 even results in frequent psychological problems such as anxiety, depression, and social isolation.⁴
47 Clinically, halitosis is categorized into genuine halitosis, pseudo-halitosis, and halitophobia.⁵ The
48 latter two types are related to psychological conditions. Only genuine halitosis is caused by
49 pathological and physiological factors. It includes intraoral halitosis (IOH) and extraoral halitosis,
50 with the former accounting for 80-90% of the cases.⁶

51 The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSCs) produced by
52 oral bacteria via complex microbe–substrate and microbe–microbe interactions and putrefaction of
53 organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and
54 periodontal disease.⁷⁻¹⁰ In particular, hydrogen sulfide (H₂S), methyl mercaptan (CH₃SH), and
55 dimethyl sulphide (C₂H₆S) are considered significant parameters and markers of halitosis.¹¹ Some
56 microorganisms, such as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella.*
57 *intermedia*, *Prevotella. nigrescens*, and *Treponema. denticola* not only do participate in periodontal
58 diseases, but they also may facilitate the production of VSC metabolism.¹² Some studies using 16S
59 rRNA amplicon sequencing and GC–MS-based metabolite profiling found that the bacterial
60 composition, diversity and metabolites of the halitosis group were different from those of the control
61 group.^{13,14} Therefore, anaerobic microbiota might play an important role in the development of
62 halitosis. Consequently, regulating the balance of the oral microbiome to reduce VSC levels is an
63 important method to treat oral malodor.

64 According to some previous reports, the current treatments for halitosis include mechanical
65 cleaning (scaling and tongue scraping) and chemical therapy (antibiotics, mouthwashes, and other
66 agents).^{15,16} However, mechanical therapy is often uncomfortable, even if carried out by the dentist. In
67 addition, although chemical therapy is generally effective for a short time, it is always associated with
68 various side effects, including the emergence of dysbacteriosis and staining of the tongue and tooth.¹⁷⁻
69 ²⁰ Consequently, new methods with fewer side-effect are constantly suggested to inhibit oral malodor.

70 As live microorganisms, probiotics confer benefits for the host when administered in appropriate
71 amounts.²¹ The beneficial effects of these probiotics are primarily related to regulating the local
72 microenvironment.^{22,23} Recently, probiotics have been widely used in the oral field.²⁴ There is a growing
73 body of evidence that the administration of probiotics might affect the composition of oral biofilms. They
74 have also been investigated in the treatment of periodontal ^{25,26} and peri-implant diseases ^{27,28}, caries ²⁹,
75 and oral candidiasis.^{30,31} Meanwhile, probiotics have also been reported as an alternative strategy to
76 relieve oral malodor.³²⁻³⁶ At present, the most common strains of probiotics in clinical studies are
77 *Lactobacillus salivarius* and *Bifidobacterium*.³⁷ To date, numerous articles have reported the
78 beneficial effects of probiotics on the treatment of halitosis.³⁸⁻⁴⁰ However, a previous systematic
79 review showed that probiotic therapy for oral malodor is associated with insufficient evidence for its
80 recommendation.⁴¹ In this review, only three included articles published during 2012-2016 and the
81 diversity of observation time might affect the reliability and quality of the results. Furthermore,

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3 82 several new studies on the efficacy of probiotics in the management of halitosis were published in 2020.
4 83 Thus, it is necessary to carry out a focused analysis of the therapeutic effects of probiotics in the treatment
5 84 of halitosis.

6 85 Therefore, this meta-analysis was undertaken to investigate the effect of probiotics on managing
7 86 halitosis from a time perspective. The results could provide some evidence for the administration of
8 87 probiotics in this field.

11 88 **METHODS**

12 89 **Patient and public involvement**

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17 90 This is a meta-analysis based on the data in the literature. It is not appropriate to involve patient and the
18 91 public in our study design and outcome measures.

19 92 **Study design**

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23 93 This systematic review was based on the recommendations of the Preferred Reporting Items for
24 94 Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO
25 95 (CRD42021227504).⁴² According to the PICOS principle, the following focused question was
26 96 structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with
27 97 placebo treatment? The populations were patients diagnosed with halitosis. The intervention was
28 98 probiotic therapy, representing the test group. The test group was compared with placebo treatment. The
29 99 considered outcomes were halitosis parameters and other indexes before and after treatment. All study
30 100 designs were RCTs.

31 101 **Search strategy**

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37 102 A critical electronic search was conducted in the bibliographic databases, mainly including PubMed,
38 103 EMBASE, Web of Science, Cochrane Central Register of Controlled Trials up to and including
39 104 February 2021 to select the published literature. Additionally, gray literature was searched in the
40 105 database System for Information on Gray literature in European and Google Scholar. The reference
41 106 lists of the included articles and some related Chinese journals were also searched manually. There
42 107 was no language restriction.

43
44 108 An initial search strategy was conducted in the PubMed with the combination of Medical Subject
45 109 Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as the follows:

46 110 Probiotic OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment

47 111 AND

48 112 halitosis OR halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris.

49 113 Endnote X7 was used for electronic title management. First, primary screening was performed
50 114 independently by two reviewers (JJL and NWH) based on the titles and abstracts. Then, the full-text
51 115 articles were used to assess the eligibility further. Any disagreement was solved by consulting a third
52 116 reviewer.

117 **Inclusion criteria**

118 Studies meeting the following conditions were considered eligible for this review: 1) study types:
119 randomized controlled clinical trials or randomized controlled cross-over studies; 2) participants:
120 systemically healthy patients diagnosed with halitosis via accepted standards (the organoleptic score
121 and or the concentration of volatile sulfur compounds); 3) interventions: evaluating the efficacy of
122 probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control
123 interventions: placebo treatment; if the control interventions included other measures, the study was
124 not included (e.g., studies comparing tongue scraping plus chlorhexidine plus probiotics and tongue
125 scraping plus chlorhexidine were excluded);³³ 5) clinical data: the measurement values, including
126 halitosis parameters and other indexes before and after treatment.

127 **Risk of bias**

128 The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for
129 randomized trials (RoB2).⁴³ This tool assesses the risk of bias in five domain areas, including
130 randomization process, deviations from intended interventions, missing outcome data, measurement
131 of outcome, and selection of the reported result. Each domain assessed bias following several
132 signaling questions. The overall bias was classified as a high risk of bias, some concerns, or a low of
133 risk of bias determined by a validated algorithm. After screening the articles, two reviewers (JJL and
134 NWH) conducted the assessment independently to reach an agreement.

135 **Data extraction**

136 Data were extracted with a researcher-designed data form with the following information: 1) basic
137 information of the included studies (first author's name and the year of publication); 2) study type
138 (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the
139 age range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and
140 frequencies); 6) clinical parameters (including the primary and secondary outcomes of final
141 participants); 7) significance and follow-up periods.

142 Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic
143 treatment ceased during the observation period, only the data before ceasing treatment were included.
144 Concerning clinical parameters, organoleptic (OLP) scores and VSC concentrations were considered
145 the primary outcomes, which were directly associated with oral malodor. The secondary outcomes in
146 this review included tongue coating scores (TCS) and plaque index (PI) because they are commonly
147 regarded as halitosis causes.

148 **Statistical analysis**

149 The statistical analysis was performed with Review Manager 5.3. All the data were group-analyzed
150 according to the follow-up time. The time ≤ 4 weeks was considered the short-term period, and
151 time > 4 weeks was considered the long-term period. In one study with three observation periods, the
152 values of 4 weeks were analyzed in the short term to keep consistent with other studies.⁴⁴ Study
153 heterogeneity was evaluated using Q statistics and the I^2 test. P-value < 0.10 was treated as the
154 standard test. When $I^2 > 50\%$ or $p < 0.10$, there was significant heterogeneity between the studies.

Subgroup or meta-regression was necessary to analyze the sources of heterogeneity. The continuous data on the halitosis parameters of the present studies were expressed with the standardized mean difference (SMD) and 95% CI (confidence interval). A random-effect model was used for analysis. Therefore, the mean difference and standard deviation had to be acquired. If the original text did not provide the related data, the mean difference could be calculated, and the standard deviation was obtained with the formula ($r_d = \sqrt{r_1^2/n_1 + r_2^2/n_2}$). The excel sheets in the articles were used to convert the values when provided with median and interquartile.^{45, 46}

RESULTS

Study selection

In total, 238 articles were potentially identified by electronic and manual searches. After eliminating the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were evaluated by reading the full texts, and seven articles met the final inclusion criteria.^{40, 44, 47-51} Figure 1 presents the whole process and reasons for exclusion.

Study characteristics

Table 1 presents the main characteristics of the included studies. In this review, all the studies were randomized control trials. The number of participants in the studies ranged between 23 and 68, with an age range of 19 to 70. Halitosis was diagnosed with OLP scores and or VSC concentrations. The probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12 weeks.

Table 1 Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score \geq 1	29 \geq 18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC \geq 1.5ng/10 mL	68 20-39	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S)	An 800-mg tablet contained 1.0 \times 10 ⁸ CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score \geq 2 VSC \geq 150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1 \times 10 ⁹ CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score $>$ 1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1 \times 10 ⁸ CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo- controlled Cross-over	OLP score \geq 1.5	23 22-67	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S) PI TCS	A tablet containing 6.7 \times 10 ⁸ CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks

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3	Penala et al.	RCT	OLP score > 2	29	OLP	A capsule mixture included	Baseline
4	(2016)	Placebo-double		25-59	PI	Lactobacillus salivarius (2×10^9	4 weeks
5		blind parallel				CFU) and Lactobacillus reuteri ($2 \times$	12 weeks
6						10^9 CFU) dissolved into 10ml	
7						distilled water to rinse for 1min,	
8						daily twice	
9	Kim et al.	RCT	OLP score ≥ 2	58	VSC (H ₂ S,	A bag of powder mixture included	Baseline
10	(2020)	Placebo-double	VSC ≥ 0.15 ng/	20-70	CH ₃ S, C ₂ H ₆ S)	Weissella. cibaria CMU (1.0×10^8	2 weeks
11		blind parallel	ml		OLP	CFU) melted in the mouth once per	4 weeks
12						day	8weeks

175 *RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU:
 176 colony forming units; H₂S: hydrogen sulfide; CH₃S: methyl mercaptan; C₂H₆S: methanliol; PI: plaque index

177 Risk of bias

178 The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five
 179 showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the
 180 OLP scores. Five articles were identified as some concerns because there were many uncertain factors
 181 in their full texts. There were only seven studies in our review; thus, a funnel plot was not performed.
 182 Figure 2 presents the concrete data on the risk of bias.

183 Primary outcomes

184 Concerning OLP, all the included studies detected the parameter with the 0-5 organoleptic scale by
 185 one or two trained and calibrated judges, and five studies contained complete data.^{40, 48-51} Studies by
 186 Keller et al. (2012) and Penala et al. (2016) reported a significant decrease in OLP in the probiotic
 187 group compared to the placebo group after treatment ($p < 0.05$). In the study by Lee et al. (2020)
 188 involving different follow-up periods, OLP scores decreased significantly in the test groups at four
 189 weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline. Additionally, the results
 190 of the other four studies indicated that the OLP scores did not differ between the two groups.

191 Concerning VSC, six articles determined VSC concentrations, with three studies detecting the
 192 values of VSC and subgroups (H₂S, CH₃SH, and C₂H₆S).^{40, 44, 50} According to the results, only two
 193 studies^{40, 50} reported a significant improvement in VSC levels in experimental groups versus placebo
 194 groups.

195 Secondary outcomes

196 Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at
 197 four weeks^{40, 49, 51}. Although a reduced tendency was observed after treatment compared with baseline
 198 values, there was no significant difference between the two groups.

199 Concerning PI, in the three studies involved,^{40, 48, 49} only one study showed a significant reduction
 200 in PI in the experimental group compared with the controlled group at 12 weeks.⁴⁸

201 Quantitative synthesis

202 A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS,
 203 and PI, according to the follow-up time. Although the detection methods of VSC were different, both
 204 of the devices exhibited similar sensitivity and specificity in the detecting of halitosis.⁵² Therefore, we

analyzed these values together. Considering the limitations of the included studies and follow-up time, the pooled estimation of TCS and PI was only performed in the short term.

In the short term, the OLP scores significantly decreased in the probiotic group compared to the control group [SMD = -0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed in VSC [SMD = -0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H₂S levels [SMD = -0.73; 95% CI (-1.36, -0.10), $p = 0.02$] (Figure 3 and Figure 5). Other items (TCS, PI, CH₃S, and C₂H₆S) were not significantly different between the experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50%$) except for H₂S levels ($I^2 = 75%$).

In the long term, there was a significant improvement in OLP scores in the experimental group [SMD = -0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 4). The results failed to show a significant difference in VSC concentrations and their subgroups levels (Figure 4 and Figure 6). The heterogeneity of VSC concentrations was substantial ($I^2 = 58%$).

DISCUSSION

Halitosis is a universal phenomenon with a negative impact on people of all ages. Most causes are related to oral health, particularly periodontal diseases and tongue coating.^{8, 53} Clinically, organoleptic test and detection of VSC concentrations are two commonly used methods to diagnose and monitor halitosis. Considering VSC generation, the use of probiotics to improve halitosis might be useful by modifying the composition of bacteria. Therefore, this review investigated the efficacy of probiotics in treating halitosis based on symptoms and causes.

This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with the placebo group regardless of the duration of observation, confirming the benefits of probiotics for halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the short term (≤ 4 weeks), with no noticeable difference in the long term (> 4 weeks). Meta-analyses were also performed in the subgroups of H₂S, CH₃SH, C₂H₆S to assess the concrete difference in VSC levels. The results showed that only H₂S levels reduced noticeably in the short term when the probiotic treatment was administered. As for TCS and PI, the meta-analysis estimated the difference based on the data of three included studies, whose observation times were all within four weeks. The results showed no significant differences between the experimental and placebo groups.

Concerning primary outcomes, OLP scores reflecting subjective perception were often treated as the gold standard for diagnosing halitosis clinically and in the research.^{54, 55} In the present article, six studies included the identified halitosis criteria of subjects with OLP scores.^{40, 44, 47-49, 51} The pooled estimation of this value was in favor of probiotic therapy rather than placebo. The VSC concentration measurement is an objective method, usually using a Halimeter or OralChroma with no significant difference. However, compared with organoleptic evaluation, VSC measurement is a quantitative variable with high sensitivity and reproducibility.⁵⁶⁻⁵⁸ The short-term results of VSC showed a significant improvement in the probiotic group compared to the placebo groups. These findings mean that probiotics might have a potential beneficial effect on relieving oral malodor symptoms in the short term. The possible mechanism is thought to be related to the oral cavity microbiome. According to some previous studies, odorous compounds are derived from the decomposition of amino acids and proteins by anaerobic bacteria.^{7, 59} The principle of probiotic therapy is the competitive inhibition of oral anaerobic bacteria to maintain balance. Based on studies on VSC and bacteria, the significantly lower VSC levels in the short term in the probiotic period might indicate the reduced activity of

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3 247 anaerobic bacteria. This finding is different from a previous study. One possible reason is the
4 248 difference in the number of included articles. Besides, the group analysis of the follow-up time might
5 249 also play a crucial role in assessing the outcomes. Meanwhile, we found that the short-term outcome
6 250 of H₂S concentration change other than CH₃SH, C₂H₆S was consistent with the total VSC. This might
7 251 be attributed to the differences in bacterial number and species related to each VSC reduction and
8 252 mechanism of probiotics^{12, 34, 60}. Additionally, the regular VSCs measurement device was reported to
9 253 be more sensitive towards H₂S than CH₃SH and C₂H₆S,⁵⁸ which is also a possible reason for the above
10 254 result. Because lower sensitivity would have a significant effect on the accurate measurement of the
11 255 relatively low VSC. However, this specific mechanism is not clear and the high heterogeneity of the
12 256 assessment reduced the reliability of the findings (p=0.04 and I²=75%).

13 257 Regarding the secondary outcomes, based on the present meta-analysis, there was no significant
14 258 difference between the experimental and placebo groups during the observation time. The possible
15 259 reason was the short observation time in the included studies because one study included in the
16 260 analysis showed a significant improvement in PI at 12 weeks.⁴⁸ Tongue coating and periodontitis are
17 261 often regarded as the leading causes of halitosis^{49, 61}. In the original articles, the TCS and PI showed a
18 262 pronounced decline after using probiotics compared with the baseline, with no decrease in the placebo
19 263 group. This phenomenon might be related to the type of probiotics, some of which were reported to
20 264 boost salivary flow by interacting with the oral microbiome.

21 265 Considering the inconsistency in the results of organoleptic scores and VSC concentrations in the
22 266 long term, time is likely to be the primary reason due to its significant effect on community diversity.
23 267 Additionally, it is also associated with the interaction of probiotics and anaerobic bacteria related to
24 268 VSC. In the present review, four articles used probiotics consisting of *Lactobacillus salivarius* as the
25 269 intervention treatment, while two articles selected *Weissella cibaria*, a bacterium isolated from
26 270 *Lactobacillus*, and one article with *Streptococcus*. The bacterial species related to VSC production
27 271 mainly included *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and
28 272 *Treponema denticola*.^{53, 62-64} *Lactobacillus salivarius* reduces the production of VSC mainly by
29 273 inhibiting the growth of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium*
30 274 *nucleatum*. However, *Streptococcus* has been reported to inhibit various bacteria, including
31 275 *Streptococcus mutans*, *Actinomyces naeslundii*, and *Rothia mucilaginosa*.³⁴ When oral bacteria vary
32 276 over time, the efficacy of probiotics, especially VSC concentrations, changes based on the number of
33 277 anaerobic bacteria. Moreover, along with VSCs, various other malodor gases are often present in bad
34 278 mouth air, such as indoles, skatole, pyridine, picolines and polyamines. The oral microbiota included
35 279 not only VSCs-producing bacteria, but also other bacteria being able to produce malodor
36 280 compounds.⁶⁵ Therefore, the long-term results may attribute to the inhibition effect of probiotics on
37 281 other bacteria. Therefore, the data about microorganisms changing in different periods are significant
38 282 for the evaluation of probiotic effects. However, only three included articles mentioned
39 283 microorganism detection. The differences in detection methods and bacterial species and insufficient
40 284 data in the included studies limited the microorganism statistical analysis in this review.

41 285 There were several limitations in the present study throughout the whole review process. First,
42 286 although both electronic and hand searches were conducted in four primary databases, it was
43 287 impossible to retrieve all the relevant studies. Second, the number of eligible studies and included
44 288 subjects was small. Third, the interventions in all the included studies included probiotics, but the
45 289 strains were different. Moreover, the doses used, frequencies, and administration periods varied
46 290 greatly. A subgroup analysis was necessary to evaluate the source of efficacy concerning the probiotic

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3 291 species, but the small size of the included articles prevented further analysis. All these factors would
4 292 inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were different.
5 293 Although there is not significant difference between them, the combined analysis might still affect the
6 294 reliability of the results. Fifth, in some included studies, the primary outcomes were presented in
7 295 different forms, such as percentages or range inter-quartiles. Finally, some important parameters,
8 296 including the microorganism species and changes, were not presented completely in some articles.
9 297 The absence of partial original data or the differences caused by data conversion equally impaired the
10 298 final results though many methods were tried to reduce the bias.

15 299 **CONCLUSION**

18 300 The present systematic review and meta-analysis indicated that probiotics might decrease the severity
19 301 of halitosis in the short term without eliminating pathogens. Considering the heterogeneity and
20 302 limitations of the study, more high-quality random clinical trials are required in the future to verify the
21 303 results.

24 304 **Contributors**

27 305 JJL collected and analyzed data, and drafted the manuscript; NWH and XHQ helped the literature
28 306 searching and statistical analysis; YZW and CZW provided help in the literature searching and figure
29 307 revises; XHQ and YKL critically reviewed the manuscript. LJL designed the experiment and critically
30 308 reviewed the manuscript; All authors agree to be accountable for the study.

33 309 **Declaration of competing Interest**

36 310 The authors declare no conflict of interest

39 311 **Ethics approval statement**

42 312 This study does not involve human participants.

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51 316 **Data availability statement**

54 317 The data supporting the findings of this study are available from the corresponding author, Longjiang
55 318 Li, upon reasonable request.

56 319

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3 450 **Figure legends**
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6 451 Figure 1: Flow diagram of literature search and inclusion.

7 452 Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for
8 453 randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and
9 454 red represent high risk of bias.

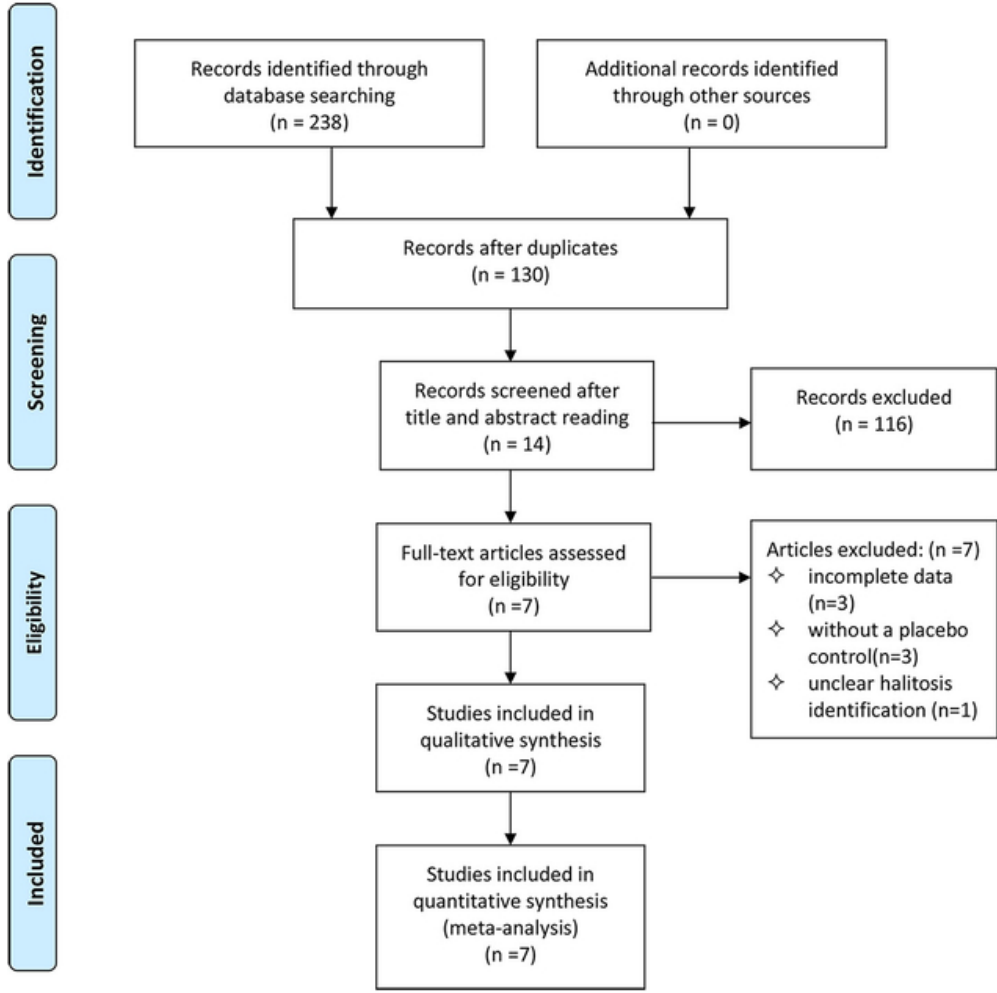
10 455 Figure 3: Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC
11 456 concentrations; (C) TCS; (D) PI.

12 457 Figure 4: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC
13 458 concentrations.

14 459 Figure 5: Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H_2S ; (B) CH_3S ; (C) $\text{C}_2\text{H}_6\text{S}$.

15 460 Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H_2S ; (B) CH_3S ; (C) $\text{C}_2\text{H}_6\text{S}$.

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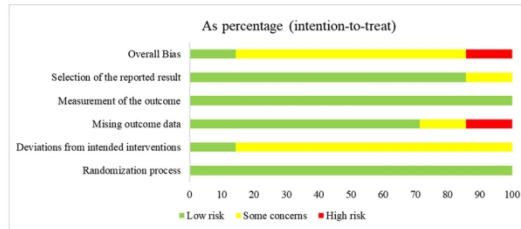
Flow diagram of literature search and inclusion.

54x58mm (300 x 300 DPI)

Intention-to-treat	Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
	1	He 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	!	!
	2	Keller 2012	probiotic	placebo	halitosis parameters	1	+	!	!	+	+	!
	3	Kim 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	4	Lee 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	5	Moussaqer 2020	probiotic	placebo	halitosis parameters	1	+	+	+	+	+	+
	6	Penala 2016	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	7	Suzuki 2014	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!

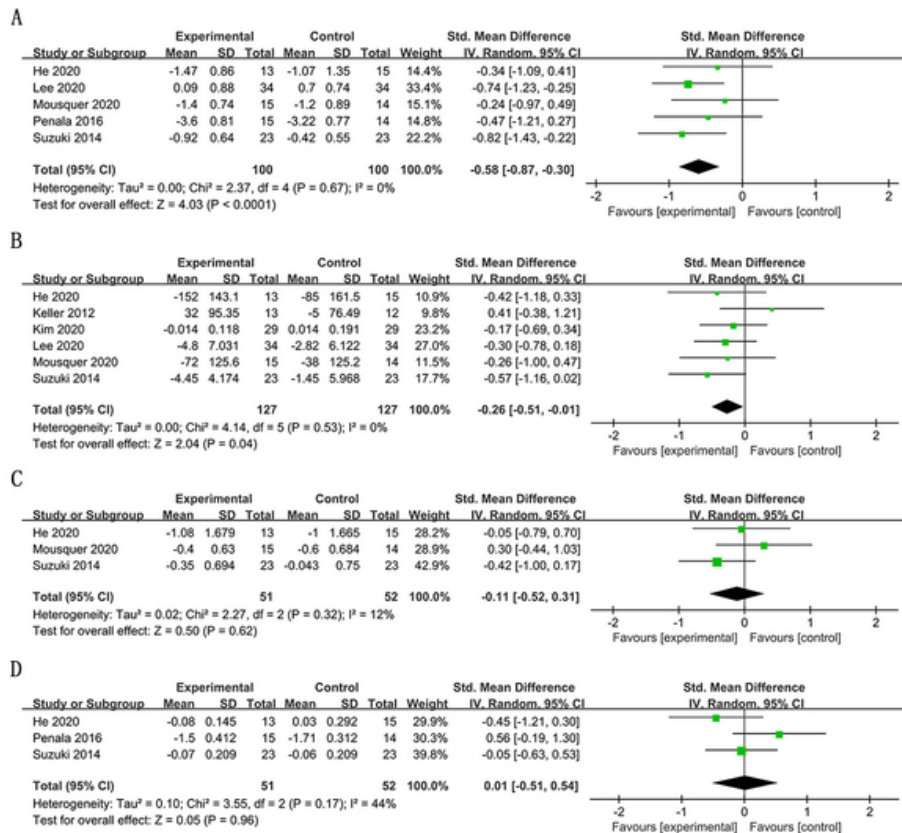
+ Low risk
! Some concerns
! High risk

D1 Randomisation process
 D2 Deviations from the intended interventions
 D3 Missing outcome data
 D4 Measurement of the outcome
 D5 Selection of the reported result



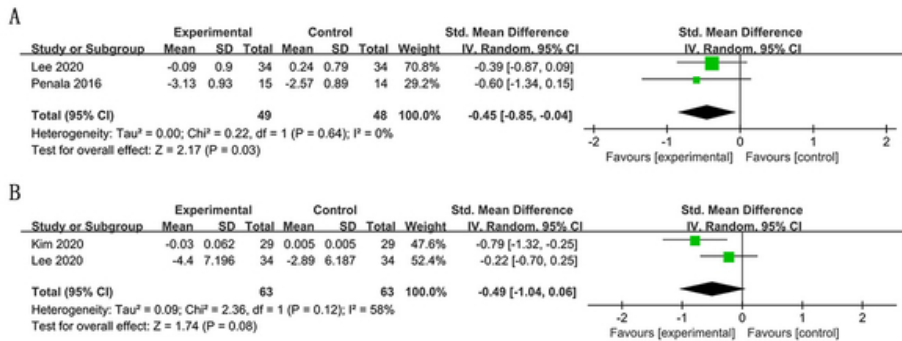
Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

166x93mm (300 x 300 DPI)



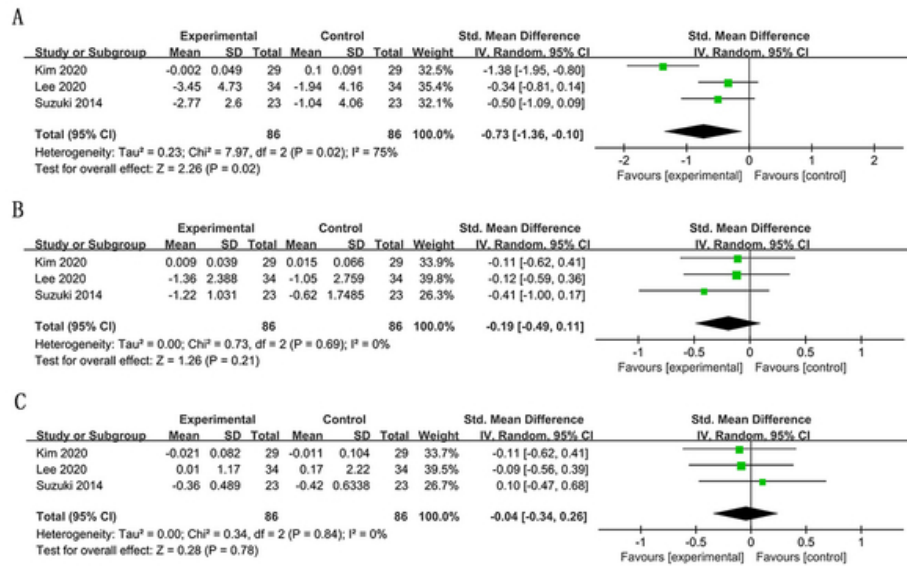
Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

53x48mm (300 x 300 DPI)



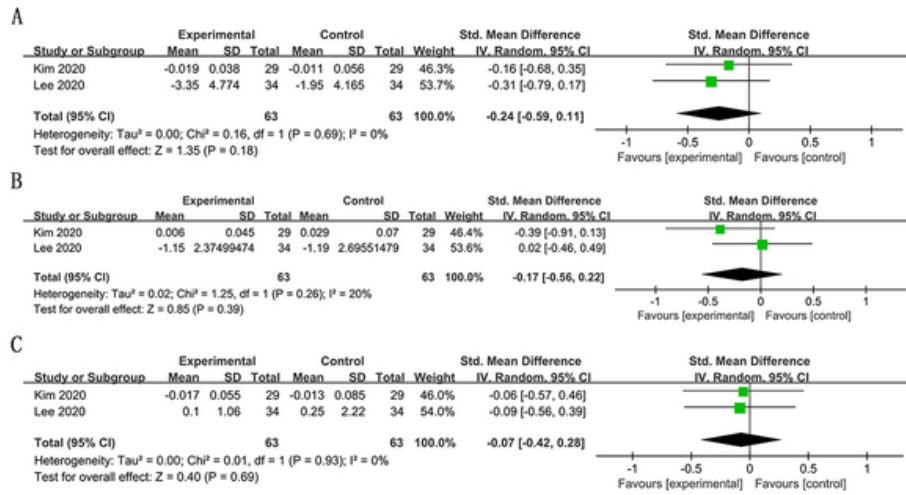
Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

54x22mm (300 x 300 DPI)



Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

53x36mm (300 x 300 DPI)



Forest plot of VSC subgroups in long-term (>4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

53x30mm (300 x 300 DPI)



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PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5



PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

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The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Primary Subject Heading:	Dentistry and oral medicine
Secondary Subject Heading:	Dentistry and oral medicine
Keywords:	Microbiology < PATHOLOGY, Infectious diseases & infestations < DERMATOLOGY, Public health < INFECTIOUS DISEASES

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Title page

Title: The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Running title: Probiotic treatment of halitosis

ABSTRACT

Background Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as an alternative method to alleviate halitosis.

Objective The present study aimed to investigate the effect of probiotics on halitosis from a time perspective.

Design and methods This is a meta-analysis study performed in indexed databases up to February 2021. Randomized controlled trials were included that compared probiotics and placebo concerning primary outcomes [organoleptic (OLP) scores and volatile sulfur compounds (VSC) levels] and secondary outcomes [tongue coating scores (TCS) and plaque index (PI)]. Data extraction and quality assessment were conducted independently by two reviewers. Publication bias and leave-one-out analyses were performed.

Results Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to synthesize data. The data were sub-grouped and analyzed in the short term (≤ 4 weeks) and long term (> 4 weeks) based on the follow-up time. Seven articles were included in this meta-analysis. Primary outcomes, both OLP scores [SMD = -0.58; 95%CI (-0.87, -0.30), $p < 0.0001$] and VSC levels [SMD = -0.26; 95%CI (-0.51, -0.01), $p = 0.04$], significantly decreased in the probiotics group compared with the placebo group in the short term. However, a significant reduction was observed only in OLP scores [SMD = -0.45; 95%CI (-0.85, -0.04), $p = 0.03$] in the long term. No significant differences were observed in secondary outcomes. There was no risk of publication bias. The leave-one-out analysis confirmed the consistency of the findings.

Conclusions According to the results of this work, it seems that probiotics (e.g., *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Streptococcus salivarius*, and *Weissella cibaria*) may relieve halitosis in the short term (≤ 4 weeks). The results of the biased assessment, limited data, and heterogeneity of clinical trials included might reduce the reliability of the conclusions.

Strengths and limitations of this study

- 39 ▶ This study included larger RCTs involved in halitosis and probiotics.
- 40 ▶ The results were rationally analyzed from the follow-up time perspective.
- 41 ▶ Subgroup analysis was done to identify the sources of heterogeneity based on the component of VSC.
- 42 ▶ The included studies had limited patients.
- 43 ▶ Some studies reported the outcomes with different forms, increasing the heterogeneity of the results.

44 INTRODUCTION

45 Halitosis, also known as “oral malodor,” is typically defined as an unpleasant odor emanating from the
46 oral cavity.¹ As a cause of patient's referral to the dentist, halitosis is the third most common disease,
47 only ranking behind dental caries and periodontal disease.² According to an epidemiological study, the
48 prevalence of halitosis is approximately 27.5% in the Chinese population.³ People have a higher demand
49 for social interactions and attach more importance to their personal image in today's society. Halitosis
50 has a significant impact on both patients' daily work and social activities and may even result in frequent
51 psychological problems such as anxiety, depression, and social isolation.⁴ Clinically, halitosis is
52 categorized into genuine halitosis, pseudo-halitosis, and halitophobia.⁵ The latter two types are related
53 to psychological conditions. Only genuine halitosis is caused by pathological and physiological factors.
54 It includes intraoral halitosis (IOH) and extraoral halitosis, with the former accounting for 80-90% of
55 the cases.⁶

56 The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSC) produced by
57 oral bacteria via complex microbe–substrate and microbe-microbe interactions and putrefaction of
58 organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and periodontal
59 disease.⁷⁻¹⁰ In particular, hydrogen sulfide (H₂S), methyl mercaptan (CH₃SH), and dimethyl sulphide
60 (C₂H₆S) are considered significant parameters and markers of halitosis.¹¹ Some microorganisms, such
61 as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella. intermedia*, *Prevotella.*
62 *nigrescens*, and *Treponema. Denticola*, not only do participate in periodontal diseases, but they also
63 may facilitate the production of VSC metabolism.¹² Some studies using 16S rRNA amplicon sequencing
64 and GC-MS-based metabolite profiling found that the bacterial composition, diversity, and metabolites
65 of the halitosis group were different from those of the control group.^{13 14} Therefore, the anaerobic oral
66 condition might play an important role in the development of halitosis. Consequently, regulating the
67 balance of the oral microbiota to reduce VSC levels is an important method to manage oral malodor.

68 The current treatments for halitosis include mechanical cleaning (scaling and tongue scraping) and
69 chemical therapy (antibiotics, mouthwashes, and other agents).^{15 16} However, mechanical therapy is
70 often uncomfortable, even if carried out by the dentist. In addition, although chemical therapy is
71 generally effective for a short time, it is always associated with various side effects, including the
72 emergence of dysbacteriosis and staining of the tongue and tooth.¹⁷⁻²⁰ Consequently, new methods with
73 fewer side effects are constantly suggested to inhibit oral malodor.

74 As live microorganisms, probiotics confer benefits to the host when administered in appropriate
75 amounts.²¹ Their beneficial effects are primarily related to regulating the local microenvironment
76 through the prevention of adhesion of pathogens and inhibition of growth of pathogens through the
77 production of bacteriocins.^{22 23} Recently, probiotics like *Lactobacillus reuteri* and *Bifidobacteria* have been
78 widely used in the oral field.²⁴ There is a growing body of evidence that the administration of probiotics
79 might affect the composition of oral biofilms. They have also been investigated in the treatment of

1
2
3 80 periodontal^{25 26} and peri-implant diseases,^{27 28} caries,²⁹ oral candidiasis^{30 31}, and oral mucositis induced by
4 81 chemo-radiotherapy.³² Meanwhile, probiotics have also been reported as an alternative strategy to relieve
5 82 oral malodor.³³⁻³⁷ However, a previous systematic review showed that probiotic therapy for oral malodor
6 83 is associated with insufficient evidence for its recommendation.³⁸ Thus, it is necessary to carry out a
7 84 focused analysis of the therapeutic effects of probiotics in the treatment of halitosis.

8 85 Therefore, this systematic review and meta-analysis was undertaken to investigate the effect of
9 86 probiotics on managing halitosis from a time perspective to provide some evidence for the
10 87 administration of probiotics in this field.

11 88 **METHODS**

12 89 **Patient and public involvement**

13 90 No patient was involved in the study.
14 91

15 92 **Study design**

16 93 This systematic review was based on the recommendations of the Preferred Reporting Items for
17 94 Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO
18 95 (CRD42021227504).³⁹ According to the PICOS principle, the following focused question was
19 96 structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with
20 97 placebo treatment? To answer our research question, we selected clinical trials according to the
21 98 following study inclusion and exclusion criteria.

22 99 **Search strategy**

23 100 A critical electronic search was conducted in the bibliographic databases, mainly including PubMed,
24 101 EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials up to and including
25 102 February 2021 to select the published literature. Additionally, gray literature was searched in the
26 103 database System for Information on Gray literature in European and Google Scholar. The reference lists
27 104 of the included articles and some related Chinese journals were also searched manually. There was no
28 105 language restriction.

29 106 An initial search strategy was conducted in PubMed with the combination of Medical Subject
30 107 Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as follows: Probiotic
31 108 OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR
32 109 halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris. The detailed search
33 110 strategy for each database is mentioned in supplemental file 1. Endnote X7 was used for electronic title
34 111 management. First, primary screening was performed independently by two reviewers (NWH and JJJ)
35 112 based on the titles and abstracts. Then, the full-text articles were used to assess the eligibility further.
36 113 Any disagreement was solved by consulting a third reviewer.

114 Study inclusion and exclusion criteria

115 The populations were patients diagnosed with halitosis. The intervention was probiotic therapy,
116 representing the experiment group. The control group was done with a placebo treatment. The considered
117 outcomes were halitosis parameters and other indexes before and after treatment. During the first stage
118 of the study selection, studies meeting the following conditions were considered eligible for this review:
119 1) study types: randomized controlled clinical trials (RCTs) or randomized controlled cross-over studies;
120 2) participants: systemically healthy patients diagnosed with halitosis via accepted standards (the
121 organoleptic scores and/or the concentrations of VSC); 3) interventions: evaluating the efficacy of
122 probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control
123 interventions: placebo treatment; 5) clinical data: the measurement values, including halitosis
124 parameters and other indexes before and after treatment. At the second stage of the selection, eligible
125 studies acquired in the first stage were identified according to the following exclusion criteria: 1) *in*
126 *vitro* and animal studies, letter to the editor, review articles, interviews, meta-analysis; 2) unclear
127 halitosis identification; 3) studies with no completed data obtained even by contacting the authors. 4)
128 interventions included other measures (e.g., studies comparing tongue scraping plus chlorhexidine plus
129 probiotics and tongue scraping plus chlorhexidine).³⁴

130 Halitosis assessment

131 The primary outcomes were evaluated for organoleptic (OLP) scores and the VSC concentration levels.
132 OLP scores reflecting subjective perception were often treated as the gold standard for diagnosing
133 halitosis clinically and in research.⁴⁰⁻⁴¹ The OLP scores were estimated by two or three evaluators (with
134 training and experience in calibrating tests). Subjects closed their mouth for 1 min and then exhaled
135 slowly from their mouth into the evaluator's nose at a distance of 10 cm. The score was evaluated
136 according to a six-point '0–5' scale (Rosenberg scale).⁴²

137 The VSC concentrations measurement is an objective method through using the Halimeter or Oral
138 Chroma with no significant difference.⁴³ Compared with organoleptic evaluation, VSC measurement is
139 a quantitative variable with high sensitivity and reproducibility.⁴⁴⁻⁴⁶ Subjects had to keep their mouth
140 closed and stop talking for 5 min before measurements. Halimeter: a beverage straw (fixed and attached
141 to the device) was inserted into the subject's mouth, located at the back of the tongue dorsum. Subjects
142 should keep their mouth slightly open and breathe through the nose. Oral Chroma: Subjects were asked
143 to keep their mouths closed for 30 s with an air-tight syringe. Then, 1 mL of mouth air was extracted
144 from the subject and injected into Oral Chroma to measure the VSC concentration.⁴⁷ Then the mean of
145 the results given by the evaluators or machines was used.

146 Risk of bias

147 The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for
148 randomized trials (RoB2).⁴⁸ This tool assesses the risk of bias in five domain areas, including
149 randomization process, deviations from intended interventions, missing outcome data, measurement of
150 outcome, and selection of the reported result. Each domain assessed bias following several signaling
151 questions. The overall bias was classified as a high risk of bias, some concerns, or a low risk of bias
152 determined by a validated algorithm. After screening the articles, two reviewers (NWH and JJJ)
153 conducted the assessment independently to reach an agreement.

154 **Data extraction**

155 Data were extracted with a researcher-designed data form with the following information: 1) basic
156 information of the included studies (first author's name and the year of publication); 2) study type
157 (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the age
158 range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and
159 frequencies); 6) clinical parameters (including the primary and secondary outcomes of final
160 participants); 7) significance and follow-up periods.

161 Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic
162 treatment ceased during the observation period, only the data before ceasing treatment were included.
163 Concerning clinical parameters, OLP scores and VSC concentrations were considered the primary
164 outcomes, directly associated with oral malodor. The secondary outcomes in this review included
165 tongue coating scores (TCS) and plaque index (PI) because they are commonly regarded as halitosis
166 causes.

167 **Statistical analysis**

168 The statistical analysis was performed with Review Manager 5.3 and Stata 12.0. All the data were
169 group-analyzed according to the follow-up time. The time ≤ 4 weeks was considered the short-term
170 period and the time > 4 weeks was considered the long-term period. In one study with three observation
171 periods, the values of 4 weeks were analyzed in the short term to keep consistent with other studies.⁴⁹
172 Study heterogeneity was evaluated using Q statistics and the I^2 test. P value < 0.10 was treated as the
173 standard test. When $I^2 > 50\%$ or p value < 0.10 , there was significant heterogeneity between the studies.⁵⁰⁻
174 ⁵² Then, subgroup analysis and sensitivity analysis were performed to analyze the sources of
175 heterogeneity. The continuous data on the halitosis parameters of the present studies were expressed
176 with the standardized mean difference (SMD) and 95% CI (confidence interval). A random-effect
177 model was used for analysis. Therefore, the mean difference and standard deviation had to be acquired.
178 If the original text did not provide the related data, the mean difference could be calculated, and the
179 standard deviation was obtained with the formula ($r_d = \sqrt{r_1^2/n_1 + r_2^2/n_2}$). The excel sheets in the
180 articles were used to convert the values when provided with median and interquartile.^{53 54} Publication
181 bias was performed subjectively by funnel plots and objectively by Egger's tests. In Egger's test, p
182 value < 0.05 indicates the presence of publication bias.⁵⁵ Sensitivity analysis (leave-one-out method)
183 was conducted to evaluate the consistency of outcomes by sequential omission of individual studies.⁵⁶

184 **RESULTS**

185 **Study selection**

186 In total, 238 articles were potentially identified by electronic and manual searches. After eliminating
187 the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were
188 evaluated by reading the full texts, and seven articles met the final inclusion criteria (Figure 1).^{42 49 57-61}

189 Study characteristics

190 Table 1 presents the main characteristics of the included studies. In this review, all the studies were
 191 randomized control trials. The number of participants in the studies ranged between 23 and 68, with an
 192 age range of 19 to 70. Halitosis was diagnosed with OLP scores and/or VSC concentrations. The
 193 probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12
 194 weeks.

195 **Table 1** Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score \geq 1	29 \geq 18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC \geq 1.5ng/10 mL	68 20-39	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S)	An 800-mg tablet contained 1.0 \times 10 ⁸ CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score \geq 2 VSC \geq 150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1 \times 10 ⁹ CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score $>$ 1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1 \times 10 ⁸ CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo- controlled Cross-over	OLP score \geq 1.5	23 22-67	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S) PI TCS	A tablet containing 6.7 \times 10 ⁸ CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks
Penala et al. (2016)	RCT Placebo-double blind parallel	OLP score $>$ 2	29 25-59	OLP PI	A capsule mixture included Lactobacillus salivarius (2 \times 10 ⁹ CFU) and Lactobacillus reuteri (2 \times 10 ⁹ CFU) dissolved into 10ml distilled water to rinse for 1min, daily twice	Baseline 4 weeks 12 weeks
Kim et al. (2020)	RCT Placebo-double blind parallel	OLP score \geq 2 VSC \geq 0.15ng/ ml	58 20-70	VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S) OLP	A bag of powder mixture included Weissella. cibaria CMU (1.0 \times 10 ⁸ CFU) melted in the mouth once per day	Baseline 2 weeks 4 weeks 8weeks

196 *RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU:
 197 colony forming units; H₂S: hydrogen sulfide; CH₃S: methyl mercaptan; C₂H₆S: methanethiol; PI: plaque index

198 Risk of bias

199 The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five
 200 showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the OLP
 201 scores. Five articles were identified as some concerns because there were many uncertain factors in
 202 their full texts. Figure 2 presents concrete data on the risk of bias.

203

204 Study outcomes

205 Primary outcomes

206 Concerning OLP, studies by Keller et al. (2012) and Penala et al. (2016) reported a significant decrease
207 in the probiotic group compared to the placebo group after treatment ($p < 0.05$).^{58,59} In the study by Lee
208 et al. (2020) involving different follow-up periods, OLP scores decreased significantly in the test groups
209 at four weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline.⁶⁰ Additionally, the
210 results of the other four studies indicated that the OLP scores did not differ between the two groups.

211 Concerning VSC, six articles determined VSC concentrations, with three studies detecting the p
212 values of VSC and subgroups (H_2S , CH_3SH , and C_2H_6S).^{49,57,60} According to the results, only two
213 studies^{57,60} reported a significant improvement in VSC levels in experimental groups versus placebo
214 groups.

215 Secondary outcomes

216 Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at four
217 weeks.^{42,57,61} Although a reduced tendency was observed after treatment compared with baseline p
218 values, there was no significant difference between the two groups.

219 Concerning PI, in the three studies involved,^{42,57,59} only one study showed a significant reduction in
220 PI in the experimental group compared with the controlled group at 12 weeks.⁵⁹

221 Quantitative synthesis

222 A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS,
223 and PI, according to the follow-up time. Although the detection methods of VSC were different, both
224 of the devices exhibited similar sensitivity and specificity in the detection of halitosis.⁴³ Therefore, we
225 analyzed these values together. Considering the limitations of the included studies and follow-up time,
226 the pooled estimation of TCS and PI was only performed in the short term.

227 In the short term, the OLP scores significantly decreased in the probiotic group compared to the
228 control group [SMD = -0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed
229 in VSC [SMD = -0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H_2S levels [SMD = -0.73; 95% CI (-1.36, -
230 0.10), $p = 0.02$]. Other items (TCS, PI, CH_3S , and C_2H_6S) were not significantly different between the
231 experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50\%$) except for H_2S
232 levels ($I^2 = 75\%$) (Figures 3 and 4).

233 In the long term, there was a significant improvement in OLP scores in the experimental group [SMD
234 = -0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 5). The results failed to show a significant difference in
235 VSC concentrations and their subgroups levels (Figures 5 and 6). The heterogeneity of VSC
236 concentrations was substantial ($I^2 = 58\%$).

237 Publication bias

238 In this systematic review and meta-analysis, we found no evidence of publication bias by the result of
239 the funnel plots and Egger's tests ($p > 0.05$) (supplementary file 2-Figures S1-S5).

240 **Sensitivity analysis**

241 Sensitivity analysis (leave-one-out method) revealed no significant change in the pooled estimation
242 when excluding any individual study (supplementary file 2-Figures S6-S9).

243

244 **DISCUSSION**

245 **Summary of the findings**

246 This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with
247 the placebo group regardless of the duration of observation, confirming the benefits of probiotics for
248 halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the
249 short term (≤ 4 weeks), with no noticeable difference in the long term (> 4 weeks). Meta-analyses were
250 also performed in the subgroups of H_2S , CH_3SH , and $\text{C}_2\text{H}_6\text{S}$ to assess the concrete difference in VSC
251 levels. The results showed that only H_2S levels reduced noticeably in the short term when the probiotic
252 treatment was administered. As for TCS and PI, the results showed no significant differences between
253 the experimental and placebo groups in the short term. There was no risk of publication bias. The
254 sensitivity analysis confirmed the consistency of the findings.

255 **Outcomes comparison and possible mechanisms**

256 Concerning the primary outcomes, in the included articles, the pooled estimation of OLP scores and
257 VSC concentrations were in favor of probiotic therapy rather than placebo in the short term.^{42 49 57-59 61}
258 The biological mechanisms may be related to the interaction between probiotics and oral microbiota.
259 According to present studies, probiotic therapy reduces odorous compound levels by inhibiting the
260 decomposition of amino acids and proteins by anaerobic bacteria.^{7 62} The significantly lower VSC
261 levels under probiotic treatment in the short term might indicate a decrease in anaerobic bacteria activity.
262 In contrast to our findings, a previous study indicated that it could not confirm the effect of probiotics
263 on reducing VSC in the short term.³⁸ The number of included articles may result in this difference.
264 However, when comes to the results in the long term, only OLP scores showed a significant reduction
265 rather than VSC concentrations. Oral microbiota contains not only VSC-producing bacteria but also
266 other bacteria capable of producing other oral malodor compounds (e.g., indoles, skatole, pyridine,
267 picolines, and polyamines).⁶³ The underlying mechanisms of the difference may result from the
268 variation and abundance of microbiota community over time, which in turn affects the efficacy of
269 probiotics, especially VSC concentration levels.^{35 49 61} Therefore, the no significant effect on VSC
270 concentrations in the long term may be due to probiotics' inhibition effect on those other bacteria.
271 Therefore, the data about microorganisms changing in different periods are significant for the evaluation
272 of probiotic effects. However, from the present studies, insufficient data in the included studies, the
273 differences in detection methods, bacterial species, and heterogeneity of clinical trials limited the
274 microorganism statistical analysis in this review.

275 Meanwhile, we found that the short-term outcome of H_2S concentration change other than CH_3SH ,
276 and $\text{C}_2\text{H}_6\text{S}$ was consistent with the total VSC. This might be related to differences in the function of
277 probiotics and in the number and species of bacteria associated with each VSC reduction.^{12 35 64}

278 Additionally, the regular VSC measurement device was reported to be more sensitive towards H₂S than
279 CH₃SH and C₂H₆S,⁴⁶ which may also account for the above result.

280 Regarding the secondary outcomes, based on the present meta-analysis, there was no significant
281 difference between the experimental and placebo groups on secondary outcomes during the observation
282 time. The possible reason was the short observation time in the included studies, as one study included
283 in the analysis showed a significant improvement in PI at 12 weeks.⁵⁹ Tongue coating and periodontitis
284 are often regarded as the leading causes of halitosis.^{42 65} However, in an original article, the TCS and
285 PI showed a pronounced decline after using probiotics compared with the baseline, with no decrease in
286 the placebo group.⁶¹ This phenomenon might be related to the type of probiotics, some of which were
287 reported to boost salivary flow by interacting with the oral microbiota.⁶⁶

288 From the current studies, there are two main types of studies on the effect of probiotics on halitosis,
289 one is to observe the effect during continuous use of probiotics and the other is to observe the effect at
290 follow-up after stopping the use of probiotics. A recently published study indicated that no significance
291 of probiotic effect was found, different from ours. The reason for the difference may be that this study
292 analyzed the collected follow-up data after stopping using probiotics for at least 2 weeks.⁶⁷ Therefore,
293 more clinical and systematic studies are needed to explore and verify the probiotic effect on the
294 management of halitosis in future research.

295 **Limitation**

296 There were several limitations in the present study throughout the whole review process. First, although
297 both electronic and hand searches were conducted in four primary databases, it was impossible to
298 retrieve all the relevant studies. Second, the number of eligible studies and included subjects was small.
299 Third, all included interventions differed in the species of probiotics, the doses used, frequencies, and
300 administration periods. A subgroup analysis was necessary to evaluate the source of efficacy concerning
301 the probiotic species, but the small size of the included articles prevented further analysis. All these
302 factors would inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were
303 different. Although there is no significant difference between them, the combined analysis might still
304 affect the reliability of the results. Fifth, in some included studies, the primary outcomes were presented
305 in different forms, such as percentages or range interquartile. Finally, some important parameters,
306 including the microorganism species and changes, were not presented completely in some articles. The
307 absence of partial original data or the differences caused by data conversion equally impaired the final
308 results though many methods were tried to reduce the bias.

309 **CONCLUSION**

310 The present systematic review and meta-analysis indicated that probiotics (e.g., *Lactobacillus salivarius*,
311 *Lactobacillus reuters*, *Streptococcus salivarius*, and *Weissella cibaria*) may ease halitosis by reducing
312 the VSC concentration levels in the short term, but there is no significant effect on the major cause of
313 halitosis such as plaque and tongue coating. Considering the heterogeneity of clinical trials included
314 and the small sample size, more high-quality random clinical trials are required in the future to verify
315 the results and to evidence the usefulness of probiotics in the management of halitosis.

316 **Contributors**

317 NWH and JLL collected and analyzed data, and drafted the manuscript; NWH and XHQ helped with
318 the literature searching and statistical analysis; YZW and CZW provided help in the literature searching
319 and figure revises; XHQ and YKL critically reviewed the manuscript. LJL designed the experiment and
320 critically reviewed the manuscript. NWH and JLL contributed equally to this paper. All authors agree
321 to be accountable for the study.

322 **Competing interests**

323 None declared.

324 **Ethics approval statement**

325 No applicable.

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329 **Data availability statement**

330 The data supporting the findings of this study are available from the corresponding author upon
331 reasonable request.

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467 Figure legends

468 Figure 1: Flow diagram of literature search strategy and inclusion, exclusion criteria.

469 Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represents a high risk of bias.

472 Figure 3: Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC

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3 473 concentrations; (C) TCS; (D) PI.

4 474 Figure 4: Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

5 475 Figure 5: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC
6 476 concentrations.

7 477 Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

8 478 Figure S1: Funnel plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC
9 479 concentrations; (C) TCS; (D) PI.

10 480 Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC
11 481 concentrations.

12 482 Figure S3: Funnel plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

13 483 Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

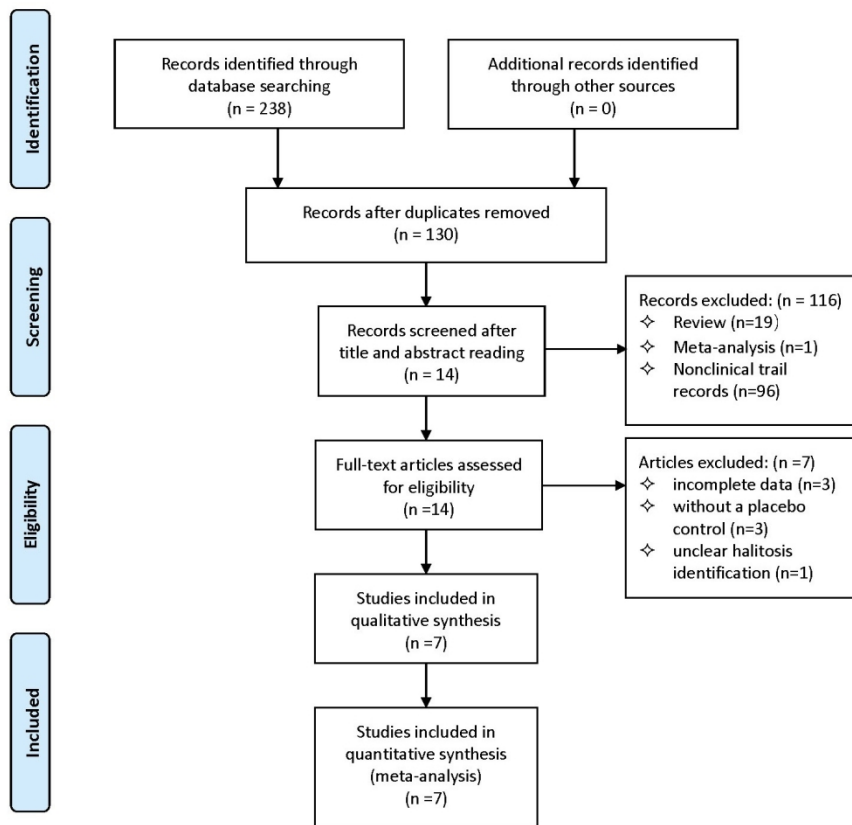
14 484 Figure S5: The result of Egger's test in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations;
15 485 (C) TCS; (D) PI; (E) H₂S; (F) CH₃S; (G) C₂H₆S.

16 486 Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B)
17 487 VSC concentrations; (C) TCS; (D) PI.

18 488 Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B)
19 489 VSC concentrations.

20 490 Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C)
21 491 C₂H₆S.

22 492 Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C)
23 493 C₂H₆S.
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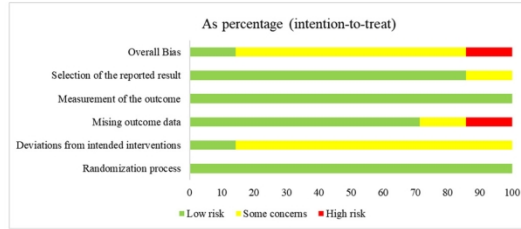


Flow diagram of literature search strategy and inclusion, exclusion criteria.

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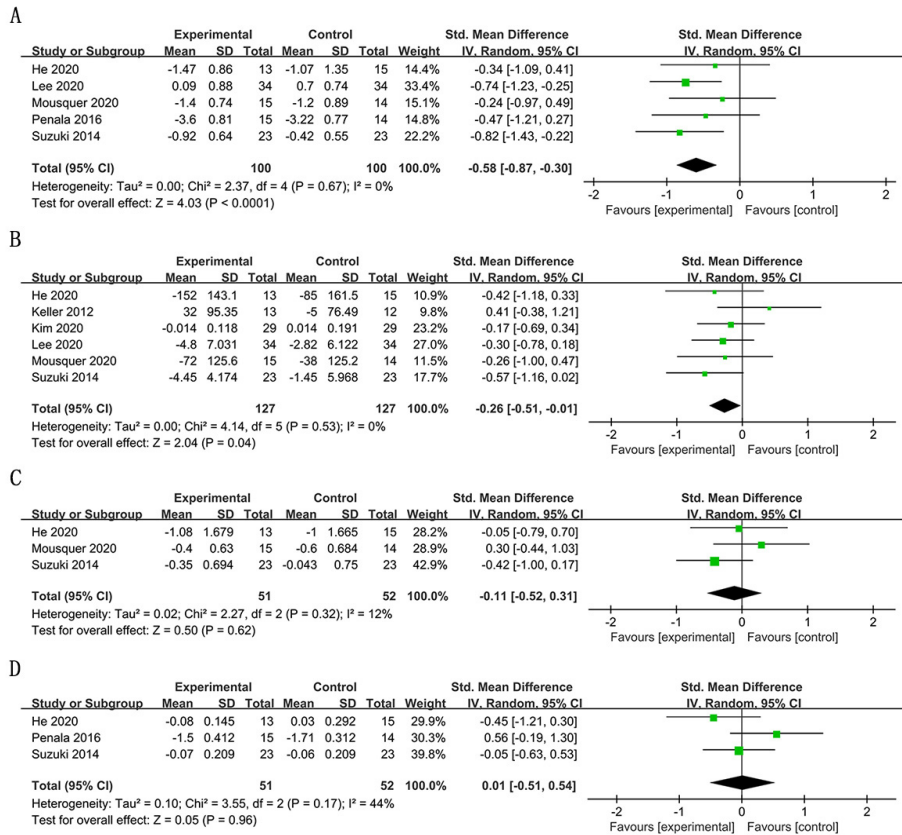
Intention-to-treat	Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
	1	He 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	!	!
	2	Keller 2012	probiotic	placebo	halitosis parameters	1	+	!	!	+	+	!
	3	Kim 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	4	Lee 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	5	Moussaqer 2020	probiotic	placebo	halitosis parameters	1	+	+	+	+	+	+
	6	Penala 2016	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	7	Suzuki 2014	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!

+ Low risk
! Some concerns
! High risk
 D1 Randomisation process
 D2 Deviations from the intended interventions
 D3 Missing outcome data
 D4 Measurement of the outcome
 D5 Selection of the reported result



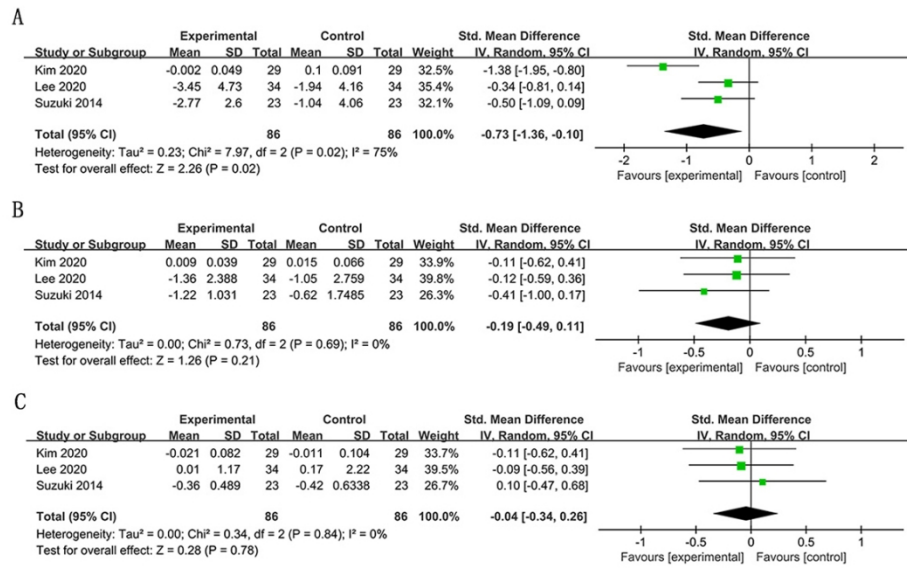
Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

167x93mm (600 x 600 DPI)



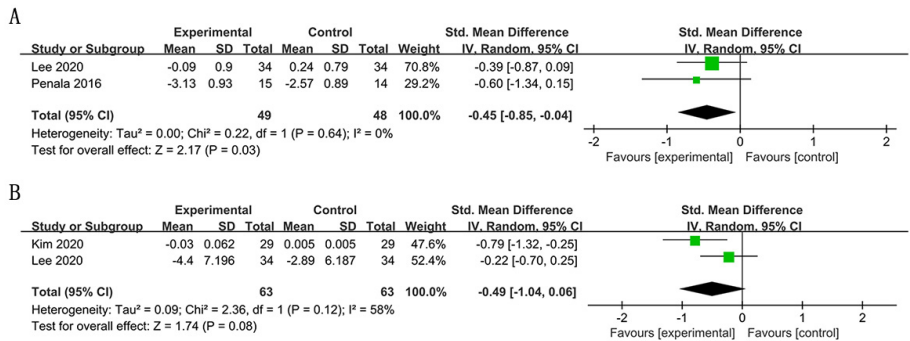
Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

108x96mm (300 x 300 DPI)



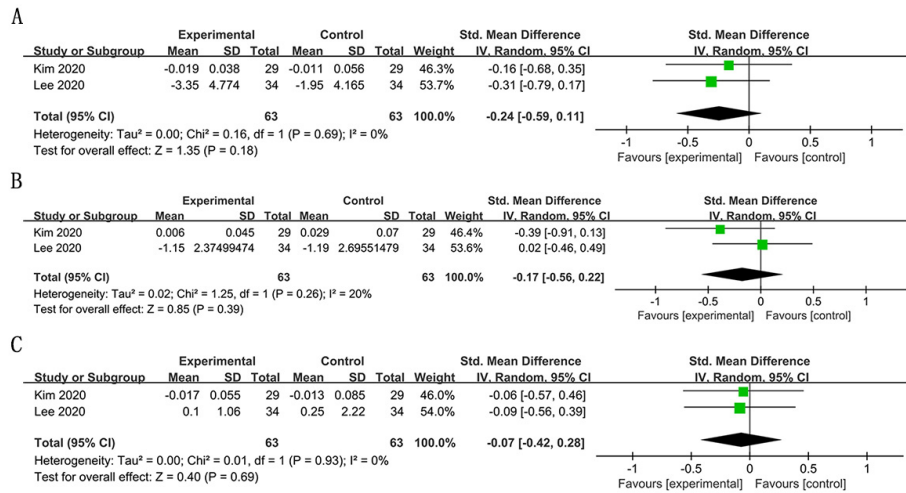
Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

107x73mm (300 x 300 DPI)



Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

108x45mm (300 x 300 DPI)



Forest plot of VSC subgroups in long-term (>4 weeks): (A) H2S; (B) CH3S; (C) C2H6S.

108x61mm (300 x 300 DPI)

Supplementary file 1

1. PubMed

Search	Query	Items found
#1	(((((Probiotic[Text Word]) OR (Probiotic[MeSH Terms])) OR (Probiotic therapy[Text Word])) OR (Probiotic effect[Text Word])) OR (Probiotic treatment[Text Word]))	27215
#2	(((((halitosis[Text Word]) OR (halitosis[MeSH Terms])) OR (malodor[Text Word])) OR (oral malodor[Text Word])) OR (malodour[Text Word])) OR (bad breath[Text Word])) OR (fedor oris[Text Word]))	2788
#3	#1 and #2	68

2. Web of science

Search	Query	Items found
#1	((TS=(Probiotic)) OR TS=(Probiotic therapy)) OR TS=(Probiotic effect)) OR TS=(Probiotic treatment)	28458
#2	(((((TS=(halitosis)) OR TS=(malodor)) OR TS=(oral malodor)) OR TS=(malodour)) OR TS=(bad breath)) OR TS=(fedor oris))	3018
#3	#1 and #2	42

3. Embase ovid search strategy

Search	Query	Items found
#1	((Probiotic or Probiotic or Probiotic therapy or Probiotic effect or Probiotic treatment) and (halitosis or halitosis or malodor or oral malodor or malodour or bad breath or fedor oris)).af.	119

4. Cochrane Central Register of Controlled Trials (CENTRAL) search strategy

Search	Query	Items found
#1	MeSH descriptor: [Halitosis] explode all trees	236
#2	(halitosis):ti,ab,kw (Word variations have been searched)	573
#3	(malodor):ti,ab,kw (Word variations have been searched)	399
#4	(oral malodor):ti,ab,kw (Word variations have been searched)	300
#5	(malodour):ti,ab,kw (Word variations have been searched)	399
#6	(bad breath):ti,ab,kw (Word variations have been searched)	258
#7	(fedor oris):ti,ab,kw (Word variations have been searched)	0
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	996
#9	MeSH descriptor: [Probiotics] explode all trees	2571

#10	(Probiotic):ti,ab,kw (Word variations have been searched)	8519
#11	(Probiotic therapy):ti,ab,kw (Word variations have been searched)	3834
#12	(Probiotic effect):ti,ab,kw (Word variations have been searched)	6398
#13	(Probiotic treatment):ti,ab,kw (Word variations have been searched)	4579
#14	#9 or #10 or #11 or #12 or #13	8603
#15	#8 and #14	8

5. Gray literature in European and Google Scholar

Search	Query	Items found
#1	Probiotic OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris	1

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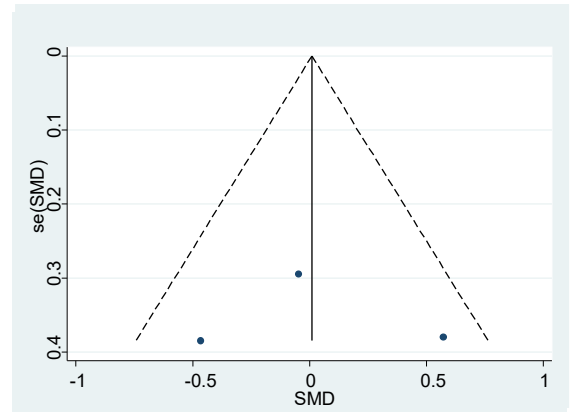
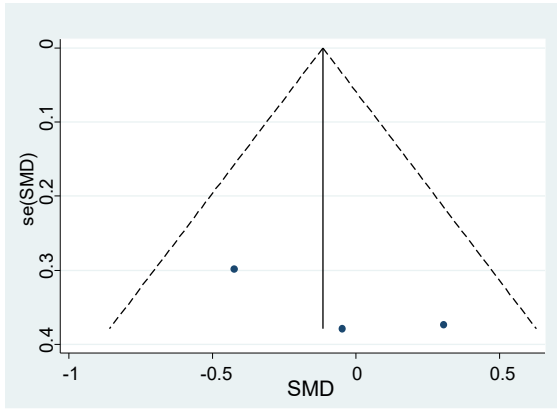
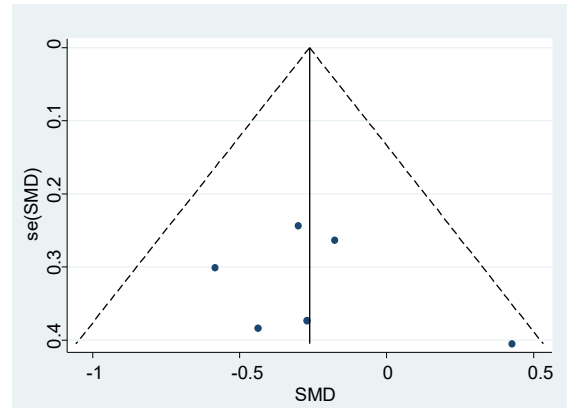
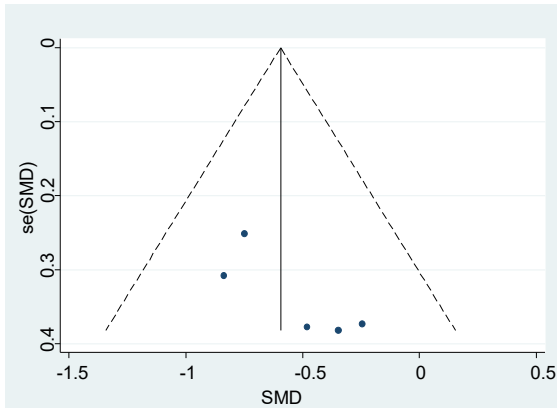


Figure S1: Funnel plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

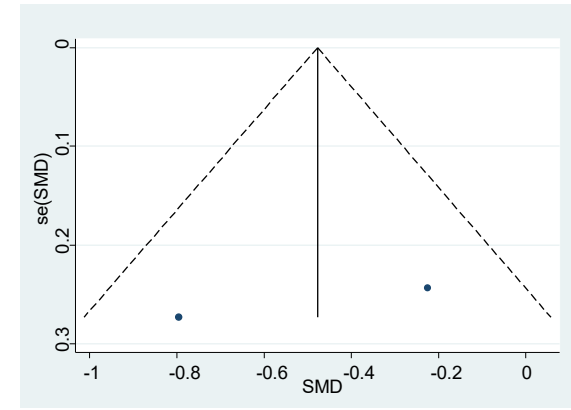
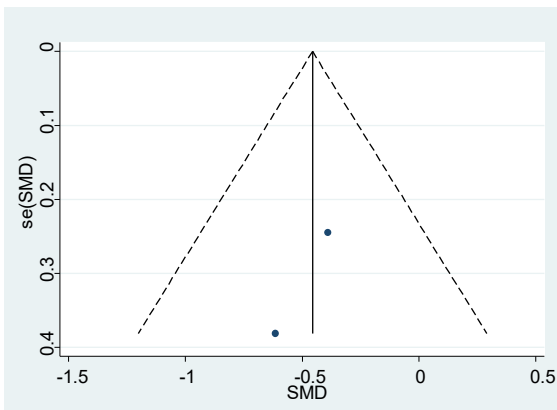
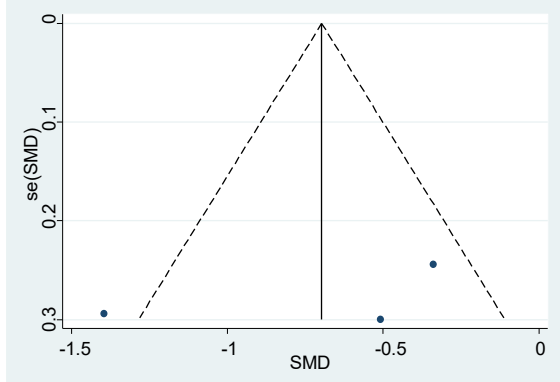


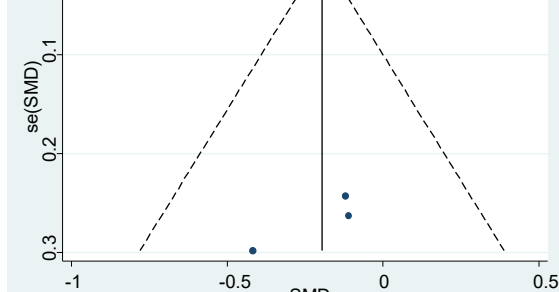
Figure S2: Funnel plot of halitosis parameters in long-term (> 4 weeks): (A) OLP scores; (B) VSC concentrations.

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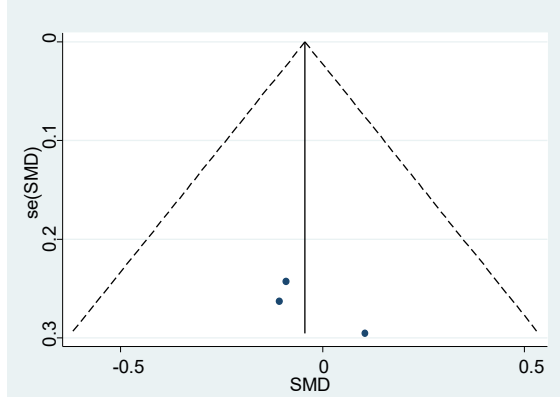
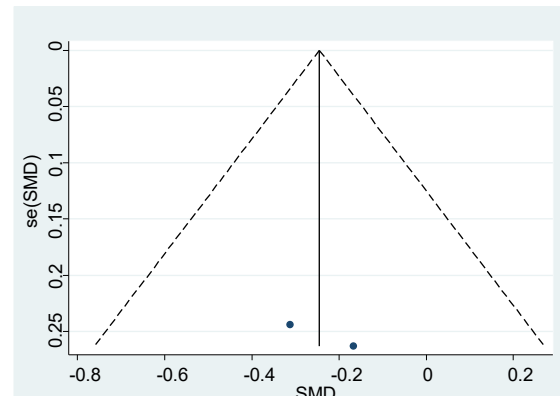


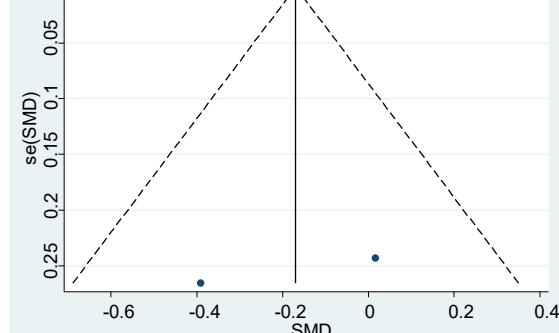
Figure S3: Funnel plot of VSC subgroups in short-term (≤ 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .

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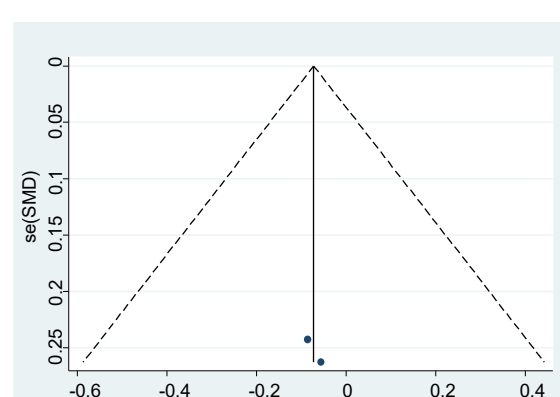


Figure S4: Funnel plot of VSC subgroups in long-term (> 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .

A

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-1.654658	.4354301	-3.80	0.032	-3.04039	-.2689248
bias	3.312431	1.341309	2.47	0.090	-.9562116	7.581074

B

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-.6982326	.6453982	-1.08	0.340	-2.490145	1.09368
bias	1.432804	2.079837	0.69	0.529	-4.341748	7.207357

C

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-2.485861	1.282746	-1.94	0.303	-18.7847	13.81297
bias	6.934603	3.728761	1.86	0.314	-40.4438	54.313

D

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-.2878511	3.056969	-0.09	0.940	-39.13033	38.55463
bias	.8711259	8.860095	0.10	0.938	-111.7071	113.4493

E

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	2.223943	3.837093	0.58	0.666	-46.53094	50.97883
bias	-10.63929	13.91572	-0.76	0.584	-187.4552	166.1766

F

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	1.252312	.6648476	1.88	0.311	-7.195378	9.700003
bias	-5.480127	2.507946	-2.19	0.273	-37.3466	26.38635

G

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-1.041425	.5483955	-1.90	0.309	-8.00945	5.926601
bias	3.788236	2.074839	1.83	0.319	-22.57509	30.15157

Figure S5: The result of Egger's test in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H₂S; (F) CH₃S; (G) C₂H₆S.

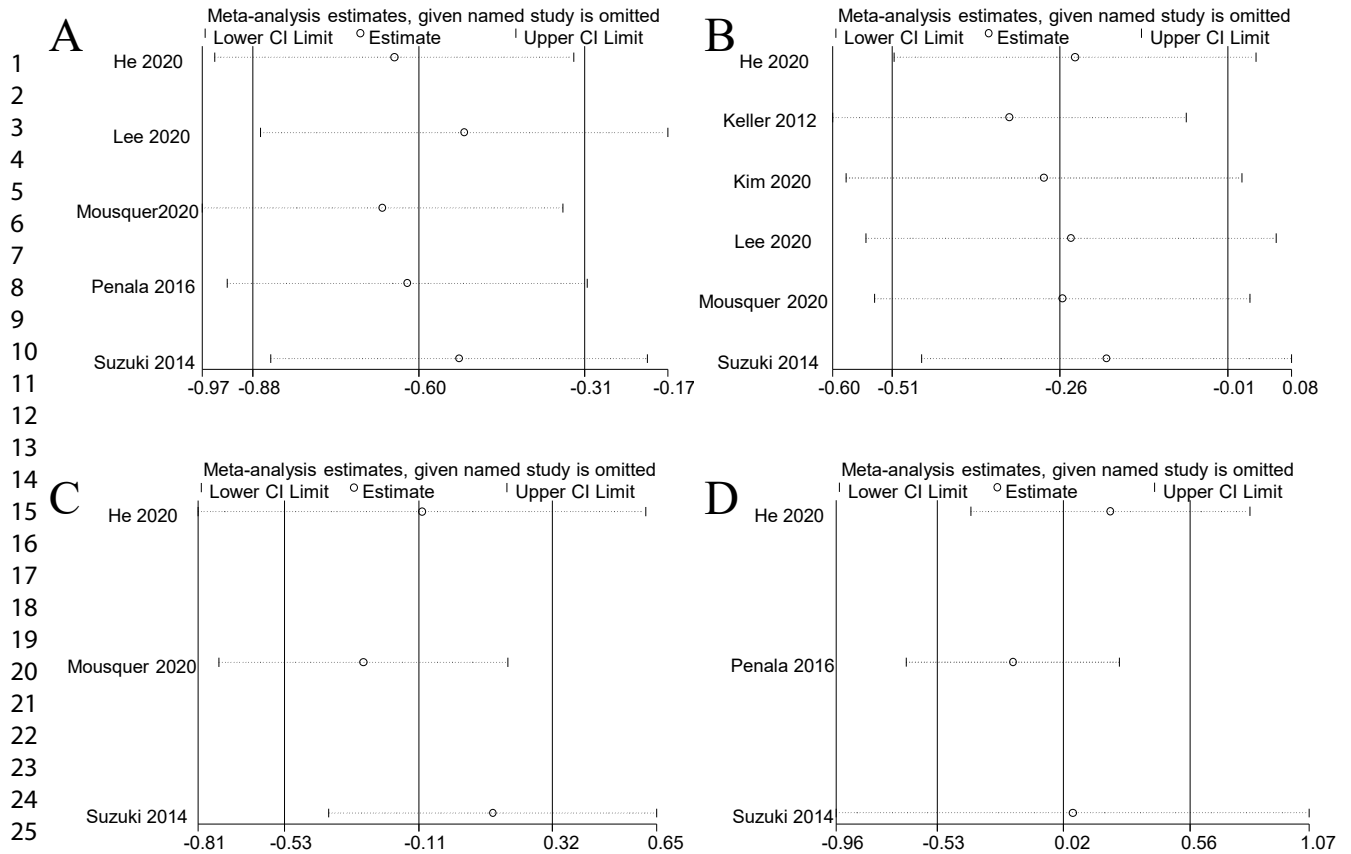


Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

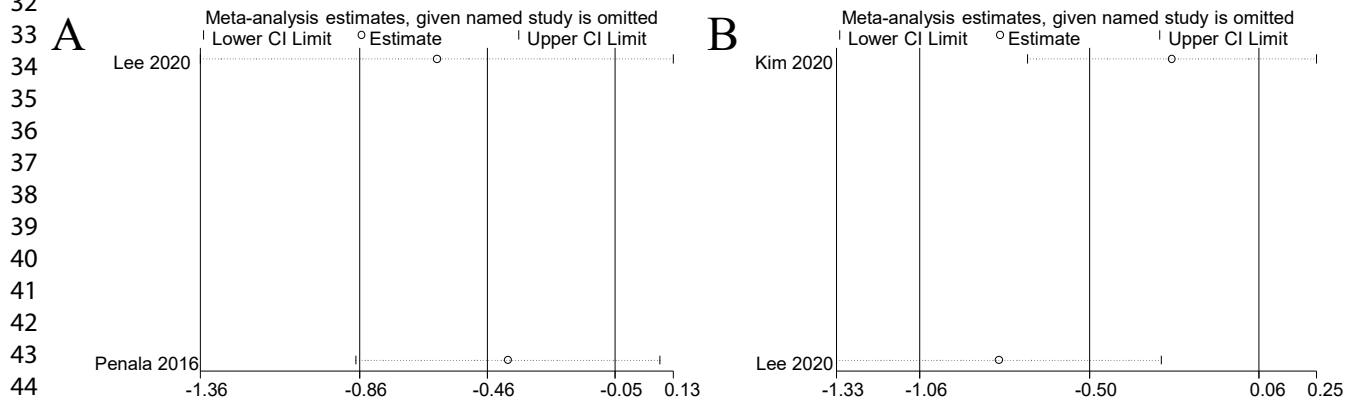


Figure S7: Sensitivity analysis of halitosis parameters in long-term (> 4 weeks): (A) OLP scores; (B) VSC concentrations.

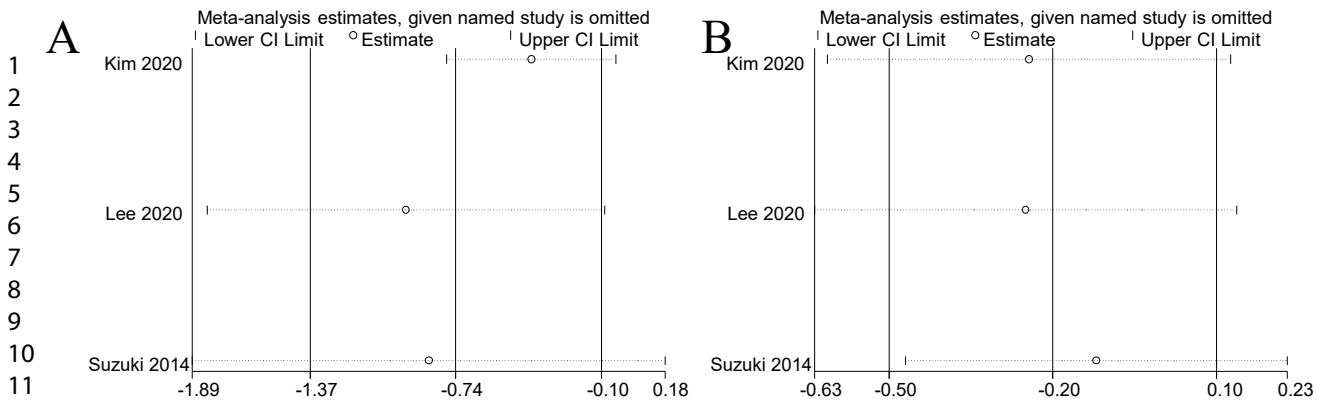


Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤ 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .

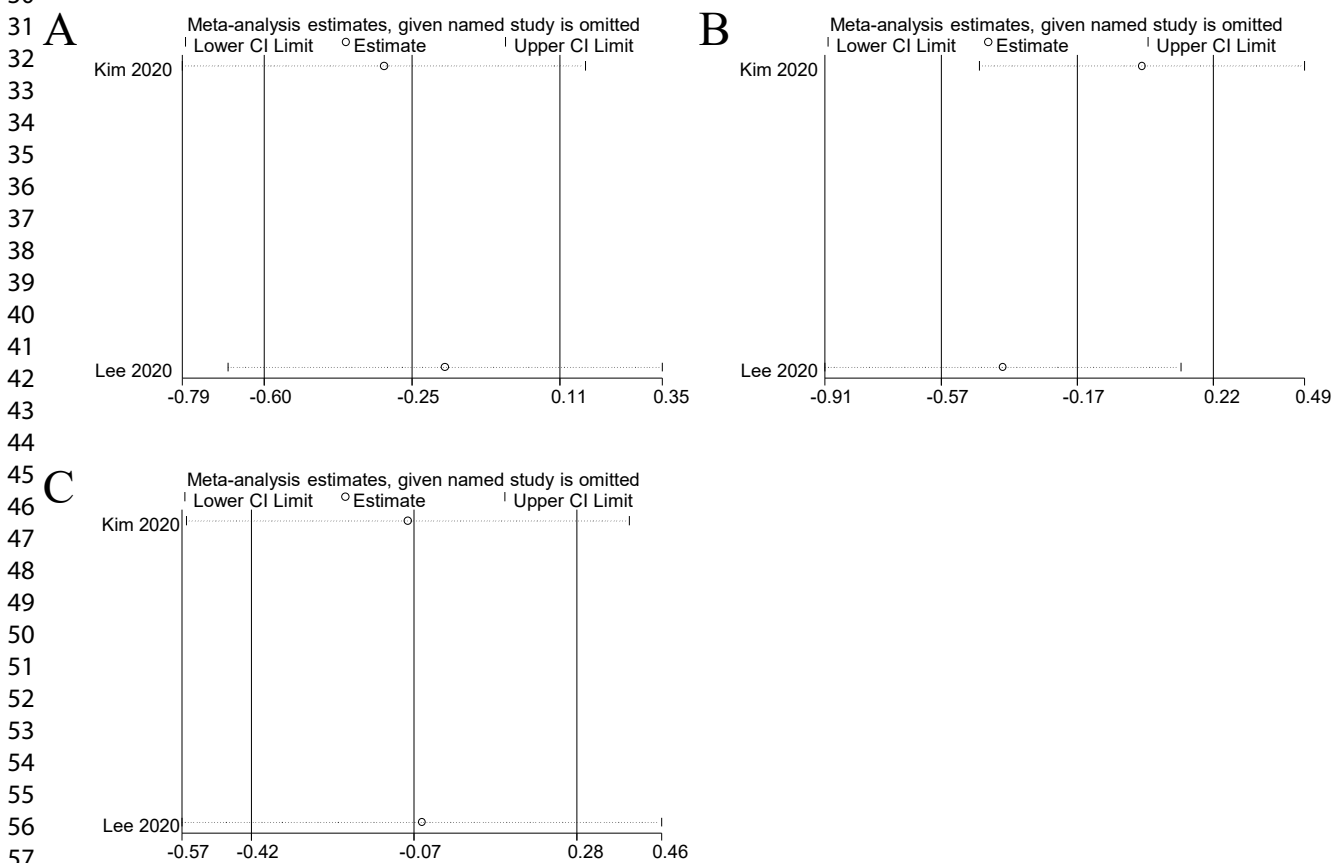


Figure S9: Sensitivity analysis of VSC subgroups in long-term (> 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .



PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5



PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

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The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis

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Title page

Title: The efficacy of probiotics in the management of halitosis: A systematic review and meta-analysis
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Running title: Probiotic treatment of halitosis

ABSTRACT

Background Halitosis is defined as a foul odor emitted from the oral cavity. Many interventions have been used to control halitosis from mouthwashes to chewing gums. Probiotics have been reported as an alternative method to alleviate halitosis.

Objective The present study aimed to investigate the effect of probiotics on halitosis from a time perspective.

Design and methods This is a meta-analysis study performed in indexed databases up to February 2021. Randomized controlled trials were included that compared probiotics and placebo concerning primary outcomes [organoleptic (OLP) scores and volatile sulfur compounds (VSC) levels] and secondary outcomes [tongue coating scores (TCS) and plaque index (PI)]. Data extraction and quality assessment were conducted independently by two reviewers. Publication bias and leave-one-out analyses were performed.

Results Standardized mean difference (SMD) and 95% confidence interval (CI) were calculated to synthesize data. The data was sub-grouped and analyzed in the short term (≤ 4 weeks) and long term (> 4 weeks) based on the follow-up time. Seven articles were included in this meta-analysis. Primary outcomes, both OLP scores [SMD = -0.58; 95%CI (-0.87, -0.30), $p < 0.0001$] and VSC levels [SMD = -0.26; 95%CI (-0.51, -0.01), $p = 0.04$], significantly decreased in the probiotics group compared with the placebo group in the short term. However, a significant reduction was observed only in OLP scores [SMD = -0.45; 95%CI (-0.85, -0.04), $p = 0.03$] in the long term. No significant differences were observed in secondary outcomes. There was no evidence of publication bias. The leave-one-out analysis confirmed that the pooled estimate was stable.

Conclusions According to the results of this work, it seems that probiotics (e.g., *Lactobacillus salivarius*, *Lactobacillus reuteri*, *Streptococcus salivarius*, and *Weissella cibaria*) may relieve halitosis in the short term (≤ 4 weeks). The results of the biased assessment, limited data, and heterogeneity of clinical trials included might reduce the reliability of the conclusions.

Strengths and limitations of this study

- 1
2
3 39 ▶ This study included larger RCTs involved in halitosis and probiotics.
4 40 ▶ The results were rationally analyzed from the follow-up time perspective.
5 41 ▶ Subgroup analysis was done to identify the sources of heterogeneity based on the component of VSC.
6 42 ▶ The included studies had limited patients.
7
8 43 ▶ Some studies reported the outcomes with different forms, increasing the heterogeneity of the results.
9

44 INTRODUCTION

45 Halitosis, also known as “oral malodor,” is typically defined as an unpleasant odor emanating from the
46 oral cavity.¹ As a cause of patient's referral to the dentist, halitosis is the third most common disease,
47 only ranking behind dental caries and periodontal disease.² According to an epidemiological study, the
48 prevalence of halitosis is approximately 27.5% in the Chinese population.³ People have a higher demand
49 for social interactions and attach more importance to their personal image in today's society. Halitosis
50 has a significant impact on both patients' daily work and social activities and may even result in frequent
51 psychological problems such as anxiety, depression, and social isolation.⁴ Clinically, halitosis is
52 categorized into genuine halitosis, pseudo-halitosis, and halitophobia.⁵ The latter two types are related
53 to psychological conditions. Only genuine halitosis is caused by pathological and physiological factors.
54 It includes intraoral halitosis (IOH) and extraoral halitosis, with the former accounting for 80-90% of
55 the cases.⁶

56 The main etiologic factor of genuine halitosis is the volatile sulfur compounds (VSC) produced by
57 oral bacteria via complex microbe–substrate and microbe-microbe interactions and putrefaction of
58 organic substrates in the oral cavity, associated with poor oral hygiene, tongue coating, and periodontal
59 disease.⁷⁻¹⁰ In particular, hydrogen sulfide (H₂S), methyl mercaptan (CH₃SH), and dimethyl sulphide
60 (C₂H₆S) are considered significant parameters and markers of halitosis.¹¹ Some microorganisms, such
61 as *Fusobacterium. nucleatum*, *Porphyromonas. gingivalis*, *Prevotella. intermedia*, *Prevotella.*
62 *nigrescens*, and *Treponema. Denticola*, not only do participate in periodontal diseases, but they also
63 may facilitate the production of VSC metabolism.¹² Some studies using 16S rRNA amplicon sequencing
64 and GC-MS-based metabolite profiling found that the bacterial composition, diversity, and metabolites
65 of the halitosis group were different from those of the control group.^{13 14} Therefore, the anaerobic oral
66 condition might play an important role in the development of halitosis. Consequently, regulating the
67 balance of the oral microbiota to reduce VSC levels is an important method to manage oral malodor.

68 The current treatments for halitosis include mechanical cleaning (scaling and tongue scraping) and
69 chemical therapy (antibiotics, mouthwashes, and other agents).^{15 16} However, mechanical therapy is
70 often uncomfortable, even if carried out by the dentist. In addition, although chemical therapy is
71 generally effective for a short time, it is always associated with various side effects, including the
72 emergence of dysbacteriosis and staining of the tongue and tooth.¹⁷⁻²⁰ Consequently, new methods with
73 fewer side effects are constantly suggested to inhibit oral malodor.

74 As live microorganisms, probiotics confer benefits to the host when administered in appropriate
75 amounts.²¹ Their beneficial effects are primarily related to regulating the local microenvironment
76 through the prevention of adhesion of pathogens and inhibition of growth of pathogens through the
77 production of bacteriocins.^{22 23} Recently, probiotics like *Lactobacillus reuteri* and *Bifidobacteria* have been
78 widely used in the oral field.²⁴ There is a growing body of evidence that the administration of probiotics
79 might affect the composition of oral biofilms. They have also been investigated in the treatment of

1
2
3 80 periodontal^{25 26} and peri-implant diseases,^{27 28} caries,²⁹ oral candidiasis^{30 31}, and oral mucositis induced by
4 81 chemo-radiotherapy.³² Meanwhile, probiotics have also been reported as an alternative strategy to relieve
5 82 oral malodor.³³⁻³⁷ However, a previous systematic review showed that probiotic therapy for oral malodor
6 83 is associated with insufficient evidence for its recommendation.³⁸ Thus, it is necessary to carry out a
7 84 focused analysis of the therapeutic effects of probiotics in the treatment of halitosis.

8 85 Therefore, this systematic review and meta-analysis was undertaken to investigate the effect of
9 86 probiotics in managing halitosis from a time perspective to provide some evidence for the
10 87 administration of probiotics in this field.

15 88 **METHODS**

19 89 **Patient and public involvement**

20 90 No patient was involved in the study.
21 91

25 92 **Study design**

26 93 This systematic review was based on the recommendations of the Preferred Reporting Items for
27 94 Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered in the PROSPERO
28 95 (CRD42021227504).³⁹ According to the PICOS principle, the following focused question was
29 96 structured: What is the clinical efficacy of probiotics in patients with halitosis when compared with
30 97 placebo treatment? To answer our research question, we selected clinical trials according to the
31 98 following study inclusion and exclusion criteria.

36 99 **Search strategy**

37 100 A critical electronic search was conducted in the bibliographic databases, mainly including PubMed,
38 101 EMBASE, Web of Science, and Cochrane Central Register of Controlled Trials up to and including
39 102 February 2021 to select the published literature. Additionally, gray literature was searched in the
40 103 database System for Information on Gray literature in European and Google Scholar. The reference lists
41 104 of the included articles and some related Chinese journals (the Chinese Journal of Stomatology, West
42 105 China Journal of Stomatology, Journal of Oral Science Research, Journal of Practical Stomatology)
43 106 were also searched manually. There was no language restriction.

44 107 An initial search strategy was conducted in PubMed with the combination of Medical Subject
45 108 Headings (Mesh) terms identified by an asterisk symbol (*) and free text words as follows: Probiotic
46 109 OR Probiotic* OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR
47 110 halitosis * OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris. The detailed search
48 111 strategy for each database was mentioned in supplemental file 1. Endnote X7 was used for electronic title
49 112 management. First, primary screening was performed independently by two reviewers (NWH and JJJ)
50 113 based on the titles and abstracts. Then, the full-text articles were used to assess the eligibility further.
51 114 Any disagreement was solved by consulting a third reviewer.

115 **Study inclusion and exclusion criteria**

116 The populations were patients diagnosed with halitosis. The intervention was probiotic therapy,
117 representing the experiment group. The control group was done with a placebo treatment. The considered
118 outcomes were halitosis parameters and other indexes before and after treatment. During the first stage
119 of the study selection, studies meeting the following conditions were considered eligible for this review:
120 1) study types: randomized controlled clinical trials (RCTs) or randomized controlled cross-over studies;
121 2) participants: systemically healthy patients diagnosed with halitosis via accepted standards (the
122 organoleptic (OLP) scores and/or the concentrations of VSC); 3) interventions: evaluating the efficacy
123 of probiotics with placebo, regardless of the probiotics species and the consumption method; 4) control
124 interventions: placebo treatment; 5) clinical data: the measurement values, including halitosis
125 parameters and other indexes before and after treatment. At the second stage of the selection, eligible
126 studies acquired in the first stage were identified according to the following exclusion criteria: 1) *in*
127 *vitro* and animal studies, letter to the editor, review articles, interviews, and meta-analyses; 2) unclear
128 halitosis identification; 3) studies with no completed data obtained even by contacting the authors. 4)
129 interventions included other measures (e.g., studies comparing tongue scraping plus chlorhexidine plus
130 probiotics and tongue scraping plus chlorhexidine).³⁴

131 **Halitosis assessment**

132 The primary outcomes were evaluated for OLP scores and the VSC concentration levels. OLP scores
133 reflecting subjective perception were often treated as the gold standard for diagnosing halitosis
134 clinically and in research.^{40 41} The OLP scores were estimated by two or three evaluators (with training
135 and experience in calibrating tests). Subjects closed their mouth for 1 min and then exhaled slowly from
136 their mouth into the evaluator's nose at a distance of 10 cm. The score was evaluated according to a
137 six-point '0–5' scale (Rosenberg scale).⁴²

138 The VSC concentrations measurement is an objective method through using the Halimeter or Oral
139 Chroma with no significant difference.⁴³ Compared with organoleptic evaluation, VSC concentrations
140 measurement is a quantitative variable with high sensitivity and reproducibility.⁴⁴⁻⁴⁶ Subjects had to
141 keep their mouth closed and stop talking for 5 min before measurements. Halimeter: a beverage straw
142 (fixed and attached to the device) was inserted into the subject's mouth, located at the back of the tongue
143 dorsum. Subjects should keep their mouth slightly open and breathe through the nose. Oral Chroma:
144 subjects were asked to keep their mouths closed for 30 s with an air-tight syringe. Then, 1 mL of mouth
145 air was extracted from the subject and injected into Oral Chroma to measure the VSC concentration.⁴⁷
146 Then the mean of the results given by the evaluators or machines was used.

147 **Risk of bias**

148 The included studies underwent a quality assessment with the Revised Cochrane risk of bias tool for
149 randomized trials (RoB2).⁴⁸ This tool assesses the risk of bias in five domain areas, including
150 randomization process, deviations from intended interventions, missing outcome data, measurement of
151 outcome, and selection of the reported result. Each domain assessed bias following several signaling
152 questions. The overall bias was classified as a high risk of bias, some concerns, or a low risk of bias
153 determined by a validated algorithm. After screening the articles, two reviewers (NWH and JJJ)
154 conducted the assessment independently to reach an agreement.

155 **Data extraction**

156 Data was extracted with a researcher-designed data form with the following information: 1) basic
157 information of the included studies (first author's name and the year of publication); 2) study type
158 (RCT); 3) diagnostic criteria for halitosis; 4) characteristics of the participants (sample volume, the age
159 range); 5) treatment (probiotic administration, including the type of bacteria, vehicles, doses, and
160 frequencies); 6) clinical parameters (including the primary and secondary outcomes of final
161 participants); 7) significance and follow-up periods.

162 Of all these variables, the follow-up periods referred to the duration of probiotic use. If probiotic
163 treatment ceased during the observation period, only the data before ceasing treatment was included.
164 Concerning clinical parameters, OLP scores and VSC concentrations were considered the primary
165 outcomes, directly associated with oral malodor. The secondary outcomes in this review included
166 tongue coating scores (TCS) and plaque index (PI) because they are commonly regarded as halitosis
167 causes.

168 **Statistical analysis**

169 The statistical analysis was performed with Review Manager 5.3 and Stata 12.0. All the data was group-
170 analyzed according to the follow-up time. The time ≤ 4 weeks was considered the short-term period and
171 the time >4 weeks was considered the long-term period. In one study with three observation periods,
172 the values of 4 weeks were analyzed in the short term to keep consistent with other studies.⁴⁹ Study
173 heterogeneity was evaluated using Q statistics and the I^2 test. P value <0.10 was treated as the standard
174 test. When $I^2 >50\%$ or p value <0.10 , there was significant heterogeneity between the studies.⁵⁰⁻⁵² Then,
175 subgroup analysis and sensitivity analysis were performed to analyze the sources of heterogeneity. The
176 continuous data on the halitosis parameters of the present studies were expressed with the standardized
177 mean difference (SMD) and 95% CI (confidence interval). A random-effect model was used for analysis.
178 Therefore, the mean difference and standard deviation had to be acquired. If the original text did not
179 provide the related data, the mean difference could be calculated, and the standard deviation was
180 obtained with the formula ($r_d = \sqrt{r_1^2/n_1 + r_2^2/n_2}$). The excel sheets in the articles were used to convert
181 the values when provided with median and interquartile.^{53 54} Publication bias was performed
182 subjectively by funnel plots and objectively by Egger's tests. In Egger's test, p value <0.05 indicates
183 the presence of publication bias.⁵⁵ Sensitivity analysis (leave-one-out method) was conducted to assess
184 the alteration by sequential omission of individual studies.⁵⁶

185 **RESULTS**

186 **Study selection**

187 In total, 238 articles were potentially identified by electronic and manual searches. After eliminating
188 the duplicates, 14 articles were included by screening the titles and abstracts. Then, these studies were
189 evaluated by reading the full texts, and seven articles met the final inclusion criteria (Figure 1).^{42 49 57-61}

190 Study characteristics

191 Table 1 presents the main characteristics of the included studies. In this review, all the studies were
 192 randomized control trials. The number of participants in the studies ranged between 23 and 68, with an
 193 age range of 19 to 70. Halitosis was diagnosed with OLP scores and/or VSC concentrations. The
 194 probiotics and placebo groups were compared, and the follow-up periods varied from two weeks to 12
 195 weeks.

196 **Table 1** Characteristics of the included studies.

Study	Type	Halitosis criterion	Subjects Age	Clinical parameters	Probiotics Administration (Vehicle, strains and frequency)	Follow-up
Mousquer et al. (2020)	RCT Placebo-double masked, parallel	OLP score \geq 1	29 \geq 18	OLP VSC TCS	A gum including 1 billion colony forming units (CFU) Lactobacillus salivarius G60 taken twice per day	Baseline 2 weeks
Lee et al. (2020)	RCT Placebo-double blind parallel	VSC \geq 1.5ng/10 mL	68 20-39	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S)	An 800-mg tablet contained 1.0 \times 10 ⁸ CFU/g Weissella cibaria taken once per day	Baseline 4 weeks 8weeks
He et al. (2020)	RCT Placebo-double blind parallel	OLP score \geq 2 VSC \geq 150ppb	28 23-44	OLP VSC TCS PI	A tablet containing 1 \times 10 ⁹ CFU Streptococcus salivarius K12 taken twice per day	Baseline 4 weeks
Keller et al. (2012)	RCT Placebo-double blind cross-over	OLP score $>$ 1	25 19-25	OLP VSC	A chewing gum containing Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 -both with a concentration of 1 \times 10 ⁸ CFU taken twice per day	Baseline 2 weeks
Suzuki et al. (2014)	RCT Double-blind placebo- controlled Cross-over	OLP score \geq 1.5	23 22-67	OLP VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S) PI TCS	A tablet containing 6.7 \times 10 ⁸ CFU Lactobacillus salivarius WB21 and 280mg xylitol taken 3 times per day	Baseline 2 weeks
Penala et al. (2016)	RCT Placebo-double blind parallel	OLP score $>$ 2	29 25-59	OLP PI	A capsule mixture included Lactobacillus salivarius (2 \times 10 ⁹ CFU) and Lactobacillus reuteri (2 \times 10 ⁹ CFU) dissolved into 10ml distilled water to rinse for 1min, daily twice	Baseline 4 weeks 12 weeks
Kim et al. (2020)	RCT Placebo-double blind parallel	OLP score \geq 2 VSC \geq 0.15ng/ ml	58 20-70	VSC (H ₂ S, CH ₃ S, C ₂ H ₆ S) OLP	A bag of powder mixture included Weissella. cibaria CMU (1.0 \times 10 ⁸ CFU) melted in the mouth once per day	Baseline 2 weeks 4 weeks 8weeks

197 *RCT: randomized controlled trials; OLP: organoleptic; VSC: volatile sulfur compounds; TCS: tongue coating scores; CFU:
 198 colony forming units; H₂S: hydrogen sulfide; CH₃S: methyl mercaptan; C₂H₆S: methanethiol; PI: plaque index

199 Risk of bias

200 The bias estimation results showed that one study had a low risk of bias, one had a high risk, and five
 201 showed some concerns. The reason for a high risk of bias was the incomplete outcome data of the OLP
 202 scores. Five articles were identified as some concerns because there were many uncertain factors in
 203 their full texts. Figure 2 presents concrete data on the risk of bias.

204

205 Study outcomes

206 Primary outcomes

207 Concerning OLP, studies by Keller et al. (2012) and Penala et al. (2016) reported a significant decrease
208 in the probiotic group compared to the placebo group after treatment ($p < 0.05$).^{58 59} In the study by Lee
209 et al. (2020) involving different follow-up periods, OLP scores decreased significantly in the test groups
210 at four weeks ($p = 0.002$) but not eight weeks ($p = 0.188$) compared to the baseline.⁶⁰ Additionally, the
211 results of the other four studies indicated that the OLP scores did not differ between the two groups.

212 Concerning VSC, six articles determined VSC concentrations, with three studies detecting the p
213 values of VSC and subgroups (H_2S , CH_3SH , and C_2H_6S).^{49 57 60} According to the results, only two
214 studies^{57 60} reported a significant improvement in VSC levels in experimental groups versus placebo
215 groups.

216 Secondary outcomes

217 Concerning TCS, three studies evaluated the changes between the probiotic and placebo groups at four
218 weeks.^{42 57 61} Although a reduced tendency was observed after treatment compared with baseline p
219 values, there was no significant difference between the two groups.

220 Concerning PI, in the three studies involved,^{42 57 59} only one study showed a significant reduction in
221 PI in the experimental group compared with the controlled group at 12 weeks.⁵⁹

222 Quantitative synthesis

223 A meta-analysis was performed including studies with similar clinical parameters of OLP, VSC, TCS,
224 and PI, according to the follow-up time. Although the detection methods of VSC were different, both
225 of the devices exhibited similar sensitivity and specificity in the detection of halitosis.⁴³ Therefore, we
226 analyzed these values together. Considering the limitations of the included studies and follow-up time,
227 the pooled estimations of TCS and PI were only performed in the short term.

228 In the short term, the OLP scores significantly decreased in the probiotic group compared to the
229 control group [SMD = -0.58; 95% CI (-0.87, -0.30), $p < 0.0001$] (Figure 3). A similar result was observed
230 in VSC [SMD = -0.26; 95% CI (-0.51, -0.01), $p = 0.04$] and H_2S levels [SMD = -0.73; 95% CI (-1.36, -
231 0.10), $p = 0.02$]. Other items (TCS, PI, CH_3S , and C_2H_6S) were not significantly different between the
232 experimental and control groups. The heterogeneity of each outcome was low ($I^2 < 50\%$) except for H_2S
233 levels ($I^2 = 75\%$) (Figures 3 and 4).

234 In the long term, there was a significant improvement in OLP scores in the experimental group [SMD
235 = -0.45; 95% CI (-0.85, -0.04), $p = 0.03$] (Figure 5). The results failed to show a significant difference in
236 VSC concentrations and their subgroups levels (Figures 5 and 6). The heterogeneity of VSC
237 concentrations was substantial ($I^2 = 58\%$).

238 Publication bias

239 In this systematic review and meta-analysis, we found no evidence of publication bias by the result of
240 the funnel plots and Egger's tests ($p > 0.05$) (supplementary file 2-Figures S1-S5).

241 Sensitivity analysis

242 Sensitivity analysis (leave-one-out method) revealed no significant change in the pooled estimation
243 when excluding any individual study (supplementary file 2-Figures S6-S9).

244

245 DISCUSSION

246 Summary of the findings

247 This meta-analysis demonstrated that probiotics significantly reduced the OLP scores compared with
248 the placebo group regardless of the duration of observation, confirming the benefits of probiotics for
249 halitosis treatment. The probiotics group exhibited a significant reduction in VSC concentrations in the
250 short term (≤ 4 weeks), with no noticeable difference in the long term (> 4 weeks). Meta-analyses were
251 also performed in the subgroups of H_2S , CH_3SH , and $\text{C}_2\text{H}_6\text{S}$ to assess the concrete difference in VSC
252 levels. The results showed that only H_2S levels reduced noticeably in the short term when the probiotic
253 treatment was administered. As for TCS and PI, the results showed no significant differences between
254 the experimental and placebo groups in the short term. There was no evidence of publication bias. The
255 sensitivity analysis confirmed that the pooled estimate was stable.

256 Outcomes comparison and possible mechanisms

257 Concerning the primary outcomes, in the included articles, the pooled estimation of OLP scores and
258 VSC concentrations were in favor of probiotic therapy rather than placebo in the short term.^{42 49 57-59 61}
259 The biological mechanisms may be related to the interaction between probiotics and oral microbiota.
260 According to present studies, probiotic therapy reduces odorous compound levels by inhibiting the
261 decomposition of amino acids and proteins by anaerobic bacteria.^{7 62} The significantly lower VSC
262 levels under probiotic treatment in the short term might indicate a decrease in anaerobic bacteria activity.
263 In contrast to our findings, a previous study indicated that it could not confirm the effect of probiotics
264 on reducing VSC in the short term.³⁸ The number of included articles may result in this difference.
265 However, when comes to the results in the long term, only OLP scores showed a significant reduction
266 rather than VSC concentrations. Oral microbiota contains not only VSC-producing bacteria but also
267 other bacteria capable of producing other oral malodor compounds (e.g., indoles, skatole, pyridine,
268 picolines, and polyamines).⁶³ The underlying mechanisms of the difference may result from the
269 variation and abundance of microbiota community over time, which in turn affects the efficacy of
270 probiotics, especially VSC concentration levels.^{35 49 61} Therefore, no significant effect on VSC
271 concentrations in the long term may be due to probiotics' inhibition effect on those other bacteria.
272 Therefore, the data about microorganisms changing in different periods are significant for the evaluation
273 of probiotic effects. However, from the present studies, insufficient data in the included studies, the
274 differences in detection methods, bacterial species, and heterogeneity of clinical trials limited the
275 microorganism statistical analysis in this review.

276 Meanwhile, we found that the short-term outcome of H_2S concentration change other than CH_3SH ,
277 and $\text{C}_2\text{H}_6\text{S}$ was consistent with the total VSC levels. This might be related to differences in the function
278 of probiotics and in the number and species of bacteria associated with each VSC reduction.^{12 35 64}

279 Additionally, the regular VSC measurement device was reported to be more sensitive towards H₂S than
280 CH₃SH and C₂H₆S,⁴⁶ which may also account for the above result.

281 Regarding the secondary outcomes, based on the present meta-analysis, there was no significant
282 difference between the experimental and placebo groups on secondary outcomes during the observation
283 time. The possible reason was the short observation time in the included studies, as one study included
284 in the analysis showed a significant improvement in PI at 12 weeks.⁵⁹ Tongue coating and periodontitis
285 are often regarded as the leading causes of halitosis.^{42 65} However, in an original article, the TCS and
286 PI showed a pronounced decline after using probiotics compared with the baseline, with no decrease in
287 the placebo group.⁶¹ This phenomenon might be related to the type of probiotics, some of which were
288 reported to boost salivary flow by interacting with the oral microbiota.⁶⁶

289 From the current studies, there are two main types of studies on the effect of probiotics on halitosis,
290 one is to observe the effect during continuous use of probiotics and the other is to observe the effect at
291 follow-up after stopping the use of probiotics. A recently published study indicated that no significance
292 of probiotic effect was found, different from ours. The reason for the difference may be that this study
293 analyzed the collected follow-up data after stopping using probiotics for at least 2 weeks.⁶⁷ In addition,
294 OLP, as the gold standard, demonstrated the efficacy of probiotics in managing halitosis. However, the
295 results of VSC concentration and subgroup analysis in the long term undermined this effect. These
296 results with various different outcomes showed the inconsistency in this study. According to Bradford-
297 Hill criteria, there would be less persuasive evidence for causation between the management of halitosis
298 and probiotics⁶⁸. Therefore, more clinical and systematic studies are needed to explore and verify the
299 probiotic effect on the management of halitosis in future research.

300 **Limitations**

301 There were several limitations in the present study throughout the whole review process. First, although
302 both electronic and hand searches were conducted in four primary databases, it was impossible to
303 retrieve all the relevant studies. Second, this study lacked persuasive evidence for causation between
304 the management of halitosis and probiotics due to the inconsistency of the pooled results. Third, all
305 included interventions differed in the species of probiotics, the doses and frequencies used, and
306 administration periods. A subgroup analysis was necessary to evaluate the source of efficacy concerning
307 the probiotic species, but the small size of the included articles prevented further analysis. All these
308 factors would inevitably affect the accuracy of outcomes. Fourth, the detection methods of VSC were
309 different. Although there is no significant difference between them, the combined analysis might still
310 affect the reliability of the results. Fifth, in some included studies, the primary outcomes were presented
311 in different forms, such as percentages or range interquartile. Finally, some important parameters,
312 including the microorganism species and changes, were not presented completely in some articles. The
313 absence of partial original data or the differences caused by data conversion equally impaired the final
314 results though many methods were tried to reduce the bias.

315 **CONCLUSION**

316 The present systematic review and meta-analysis indicated that probiotics (e.g., *Lactobacillus salivarius*,
317 *Lactobacillus reuters*, *Streptococcus salivarius*, and *Weissella cibaria*) may ease halitosis by reducing
318 the VSC concentration levels in the short term, but there is no significant effect on the major cause of
319 halitosis such as plaque and tongue coating. Considering the heterogeneity of clinical trials included

320 and the small sample size, more high-quality random clinical trials are required in the future to verify
321 the results and to evidence the efficacy of probiotics in the management of halitosis.

322 **Contributors**

323 NWH and JJJ collected and analyzed data, and drafted the manuscript; NWH and XHQ helped with
324 the literature searching and statistical analysis; YZW and CZW provided help in the literature searching
325 and figure revises; XHQ and YKL critically reviewed the manuscript. LJJ designed the experiment and
326 critically reviewed the manuscript. NWH and JJJ contributed equally to this paper. All authors agree
327 to be accountable for the study.

328 **Competing interests**

329 None declared.

330 **Ethics approval statement**

331 No applicable.

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335 **Data availability statement**

336 The data supporting the findings of this study are available from the corresponding author upon
337 reasonable request.

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3 474 **Figure legends**
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6 475 Figure 1: Flow diagram of literature search strategy and inclusion, exclusion criteria.

7 476 Figure 2: Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for
8 477 randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red
9 478 represents a high risk of bias.

10 479 Figure 3: Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC
11 480 concentrations; (C) TCS; (D) PI.

12 481 Figure 4: Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

13 482 Figure 5: Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC
14 483 concentrations.

15 484 Figure 6: Forest plot of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

16 485 Figure S1: Funnel plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC
17 486 concentrations; (C) TCS; (D) PI.

18 487 Figure S2: Funnel plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC
19 488 concentrations.

20 489 Figure S3: Funnel plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

21 490 Figure S4: Funnel plot of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

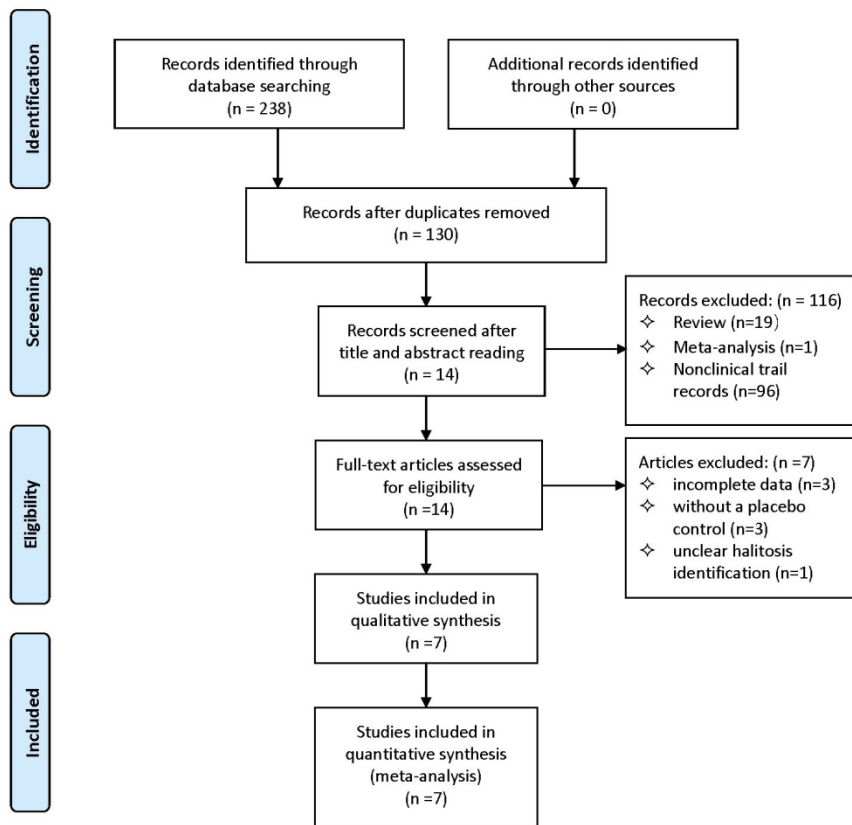
22 491 Figure S5: The result of Egger's test in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations;
23 492 (C) TCS; (D) PI; (E) H₂S; (F) CH₃S; (G) C₂H₆S.

24 493 Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B)
25 494 VSC concentrations; (C) TCS; (D) PI.

26 495 Figure S7: Sensitivity analysis of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B)
27 496 VSC concentrations.

28 497 Figure S8: Sensitivity analysis of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C)
29 498 C₂H₆S.

30 499 Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C)
31 500 C₂H₆S.
32 501

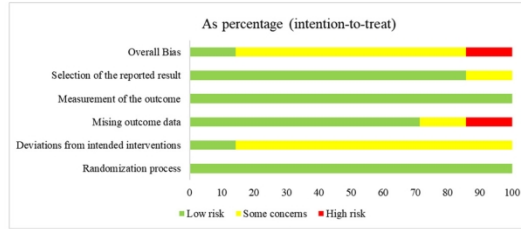


Flow diagram of literature search strategy and inclusion, exclusion criteria.

142x118mm (300 x 300 DPI)

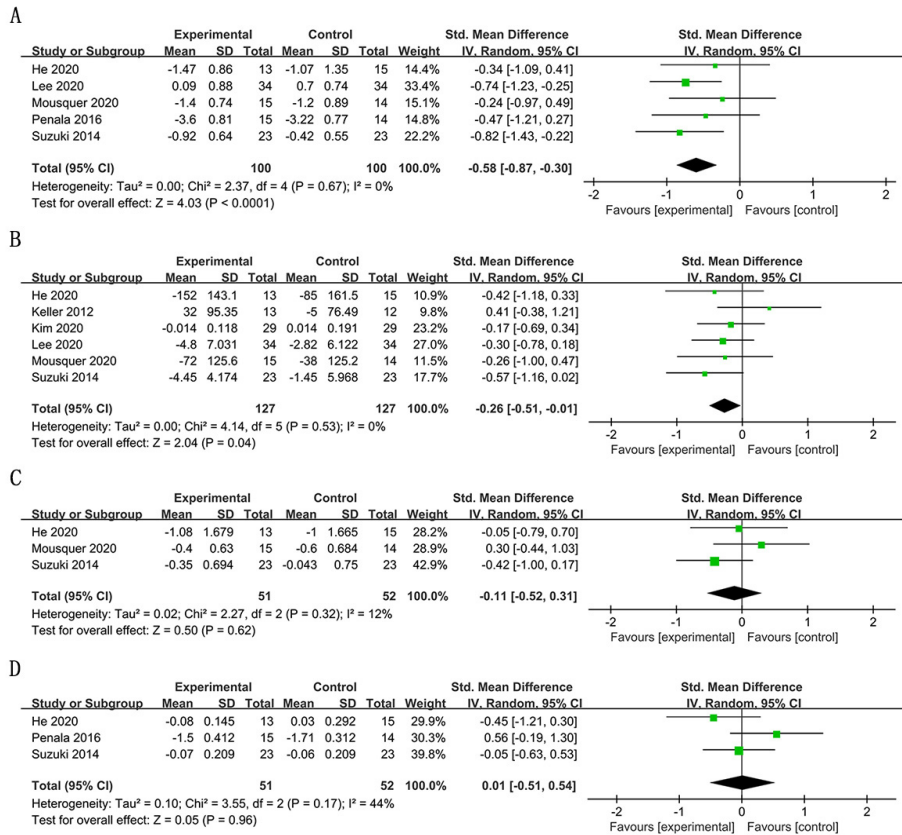
Intention-to-treat	Unique ID	Study ID	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
	1	He 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	!	!
	2	Keller 2012	probiotic	placebo	halitosis parameters	1	+	!	!	+	+	!
	3	Kim 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	4	Lee 2020	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	5	Moussaqer 2020	probiotic	placebo	halitosis parameters	1	+	+	+	+	+	+
	6	Penala 2016	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!
	7	Suzuki 2014	probiotic	placebo	halitosis parameters	1	+	!	+	+	+	!

+ Low risk
! Some concerns
! High risk
 D1 Randomisation process
 D2 Deviations from the intended interventions
 D3 Missing outcome data
 D4 Measurement of the outcome
 D5 Selection of the reported result



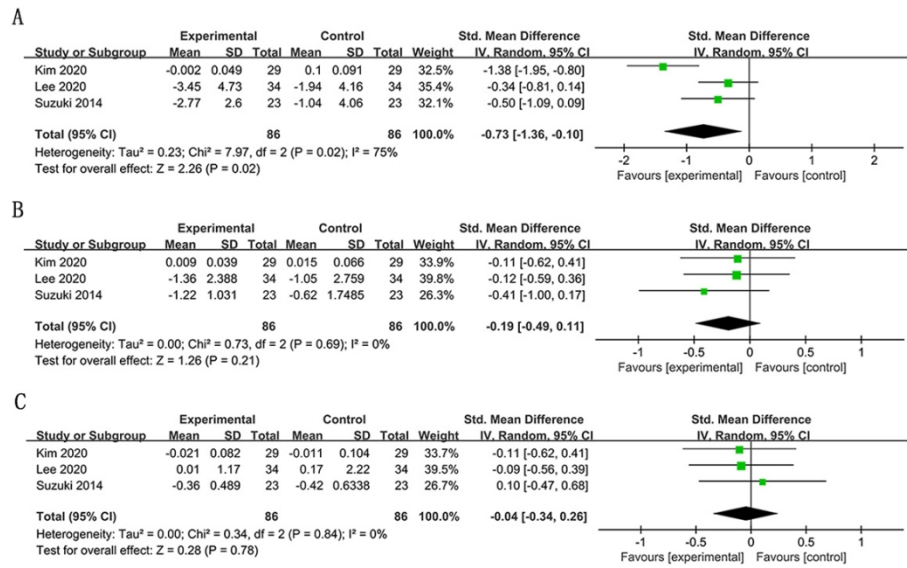
Quality assessment of the selected studies (the Revised Cochrane risk of bias tool for randomized trials (RoB2)). Green represents low risk of bias, yellow represents some concerns and red represent high risk of bias.

167x93mm (600 x 600 DPI)



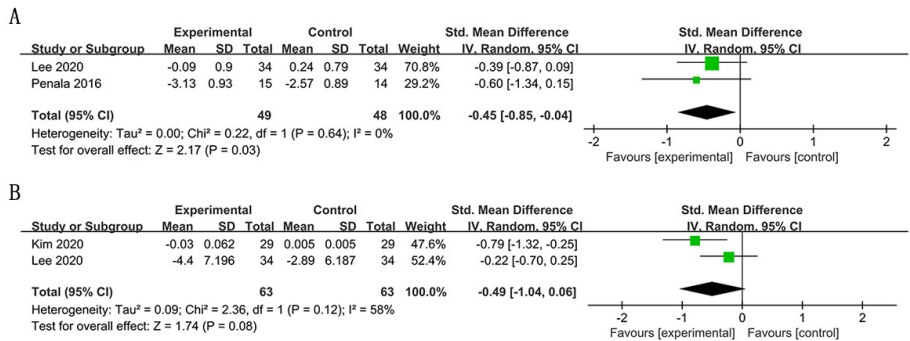
Forest plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

108x96mm (300 x 300 DPI)



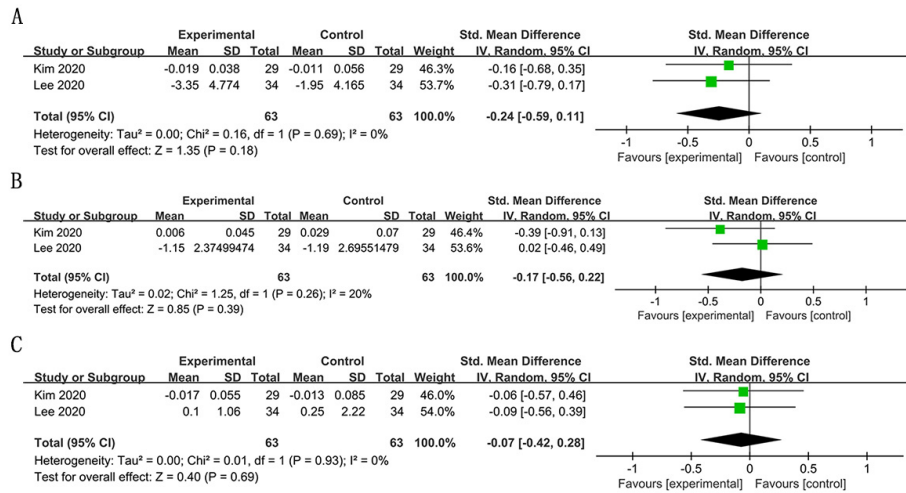
Forest plot of VSC subgroups in short-term (≤ 4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

107x73mm (300 x 300 DPI)



Forest plot of halitosis parameters in long-term (>4 weeks): (A) OLP scores; (B) VSC concentrations.

108x45mm (300 x 300 DPI)



Forest plot of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

108x61mm (300 x 300 DPI)

Supplementary file 1

1. PubMed

Search	Query	Items found
#1	(((((Probiotic[Text Word]) OR (Probiotic[MeSH Terms])) OR (Probiotic therapy[Text Word])) OR (Probiotic effect[Text Word])) OR (Probiotic treatment[Text Word]))	27215
#2	(((((halitosis[Text Word]) OR (halitosis[MeSH Terms])) OR (malodor[Text Word])) OR (oral malodor[Text Word])) OR (malodour[Text Word])) OR (bad breath[Text Word])) OR (fedor oris[Text Word]))	2788
#3	#1 and #2	68

2. Web of science

Search	Query	Items found
#1	((TS=(Probiotic)) OR TS=(Probiotic therapy)) OR TS=(Probiotic effect)) OR TS=(Probiotic treatment)	28458
#2	((TS=(halitosis)) OR TS=(malodor)) OR TS=(oral malodor)) OR TS=(malodour)) OR TS=(bad breath)) OR TS=(fedor oris)	3018
#3	#1 and #2	42

3. Embase ovid search strategy

Search	Query	Items found
#1	((Probiotic or Probiotic or Probiotic therapy or Probiotic effect or Probiotic treatment) and (halitosis or halitosis or malodor or oral malodor or malodour or bad breath or fedor oris)).af.	119

4. Cochrane Central Register of Controlled Trials (CENTRAL) search strategy

Search	Query	Items found
#1	MeSH descriptor: [Halitosis] explode all trees	236
#2	(halitosis):ti,ab,kw (Word variations have been searched)	573
#3	(malodor):ti,ab,kw (Word variations have been searched)	399
#4	(oral malodor):ti,ab,kw (Word variations have been searched)	300
#5	(malodour):ti,ab,kw (Word variations have been searched)	399
#6	(bad breath):ti,ab,kw (Word variations have been searched)	258
#7	(fedor oris):ti,ab,kw (Word variations have been searched)	0
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	996
#9	MeSH descriptor: [Probiotics] explode all trees	2571

#10	(Probiotic):ti,ab,kw (Word variations have been searched)	8519
#11	(Probiotic therapy):ti,ab,kw (Word variations have been searched)	3834
#12	(Probiotic effect):ti,ab,kw (Word variations have been searched)	6398
#13	(Probiotic treatment):ti,ab,kw (Word variations have been searched)	4579
#14	#9 or #10 or #11 or #12 or #13	8603
#15	#8 and #14	8

5. Gray literature in European and Google Scholar

Search	Query	Items found
#1	Probiotic OR Probiotic therapy OR Probiotic effect OR Probiotic treatment AND halitosis OR malodor OR oral malodor OR malodour OR bad breath OR fetor oris	1

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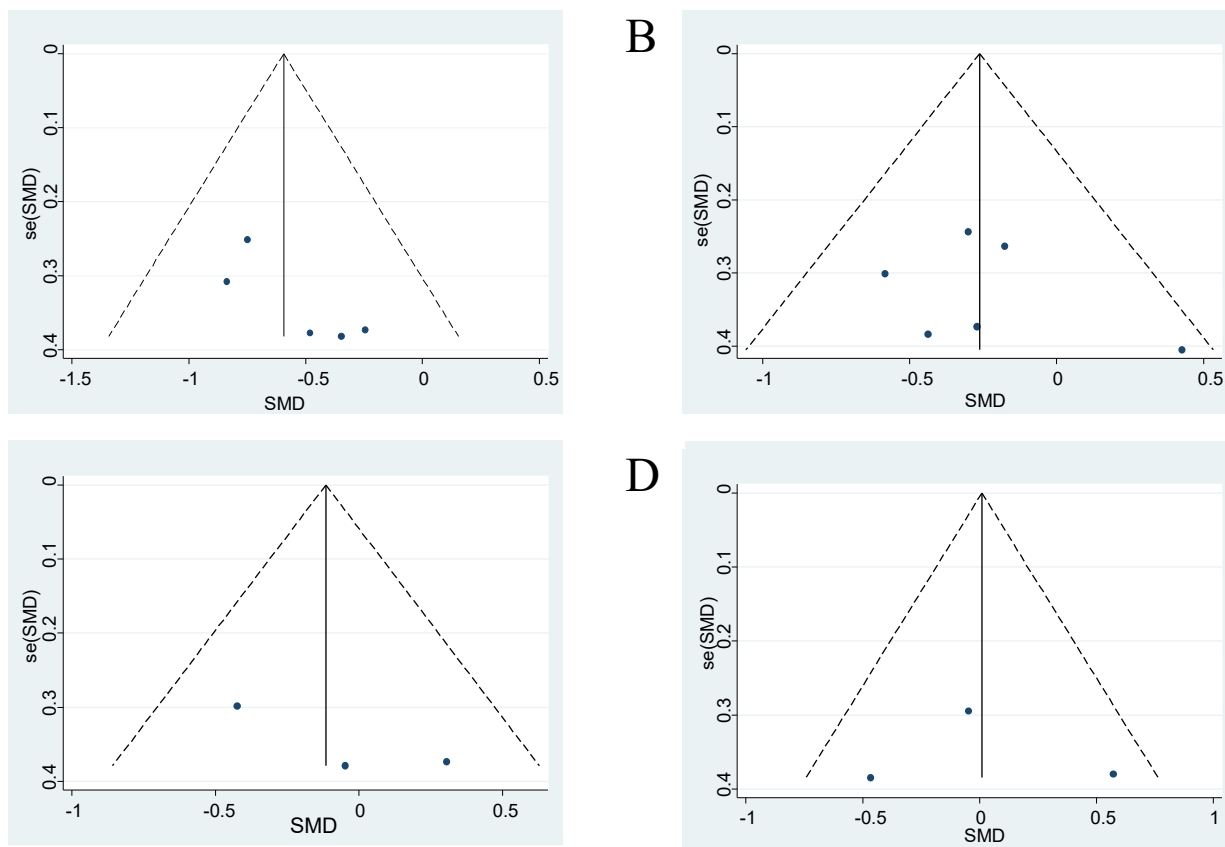


Figure S1: Funnel plot of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

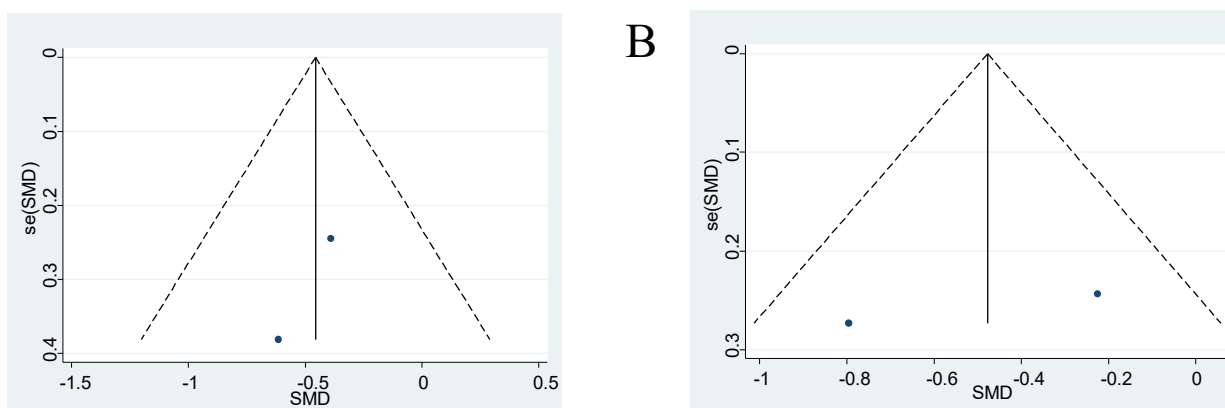
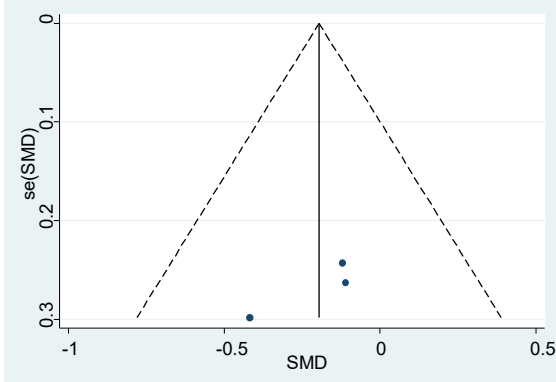
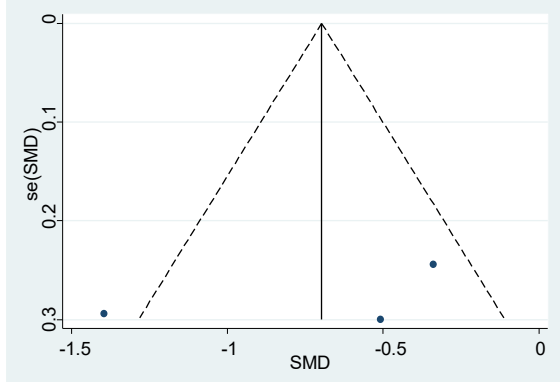


Figure S2: Funnel plot of halitosis parameters in long-term (> 4 weeks): (A) OLP scores; (B) VSC concentrations.

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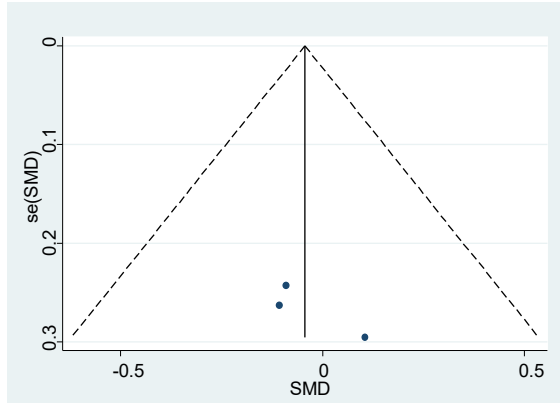
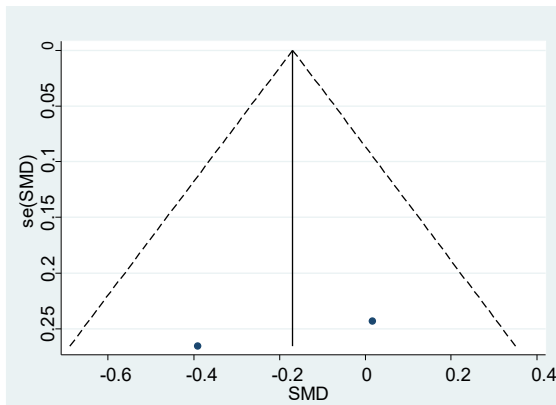
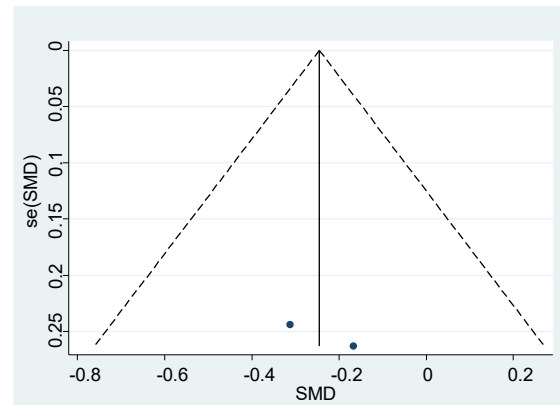


Figure S3: Funnel plot of VSC subgroups in short-term (≤ 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .

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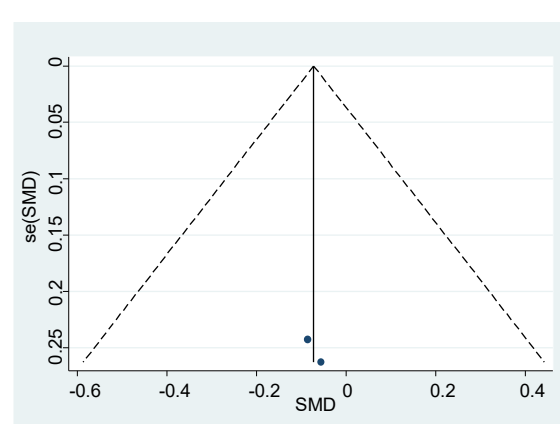


Figure S4: Funnel plot of VSC subgroups in long-term (> 4 weeks): (A) H_2S ; (B) CH_3S ; (C) C_2H_6S .

A

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-1.654658	.4354301	-3.80	0.032	-3.04039	-.2689248
bias	3.312431	1.341309	2.47	0.090	-.9562116	7.581074

B

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-.6982326	.6453982	-1.08	0.340	-2.490145	1.09368
bias	1.432804	2.079837	0.69	0.529	-4.341748	7.207357

C

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-2.485861	1.282746	-1.94	0.303	-18.7847	13.81297
bias	6.934603	3.728761	1.86	0.314	-40.4438	54.313

D

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-.2878511	3.056969	-0.09	0.940	-39.13033	38.55463
bias	.8711259	8.860095	0.10	0.938	-111.7071	113.4493

E

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	2.223943	3.837093	0.58	0.666	-46.53094	50.97883
bias	-10.63929	13.91572	-0.76	0.584	-187.4552	166.1766

F

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	1.252312	.6648476	1.88	0.311	-7.195378	9.700003
bias	-5.480127	2.507946	-2.19	0.273	-37.3466	26.38635

G

Egger's test						
Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-1.041425	.5483955	-1.90	0.309	-8.00945	5.926601
bias	3.788236	2.074839	1.83	0.319	-22.57509	30.15157

Figure S5: The result of Egger's test in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI; (E) H₂S; (F) CH₃S; (G) C₂H₆S.

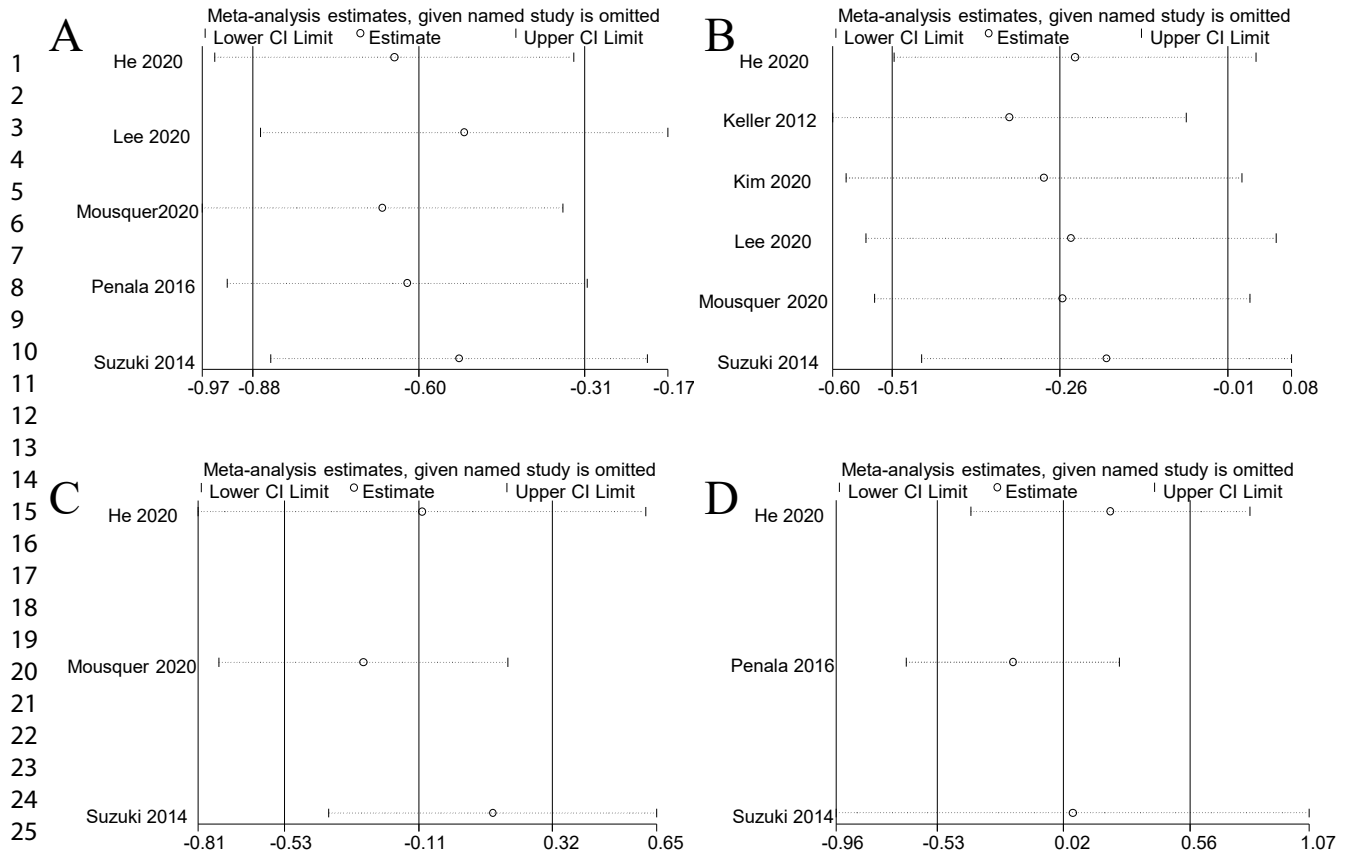


Figure S6: Sensitivity analysis of halitosis parameters in short-term (≤ 4 weeks): (A) OLP scores; (B) VSC concentrations; (C) TCS; (D) PI.

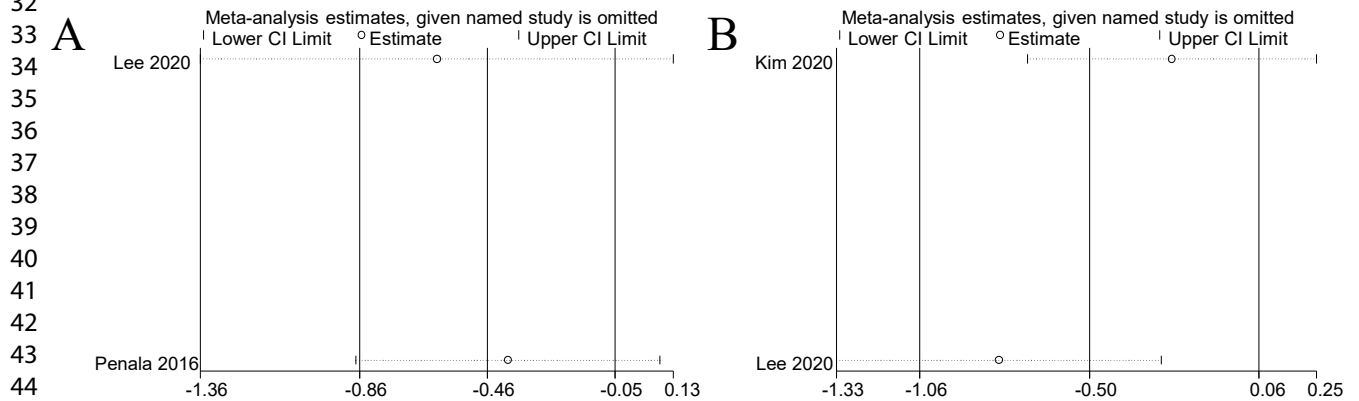


Figure S7: Sensitivity analysis of halitosis parameters in long-term (> 4 weeks): (A) OLP scores; (B) VSC concentrations.

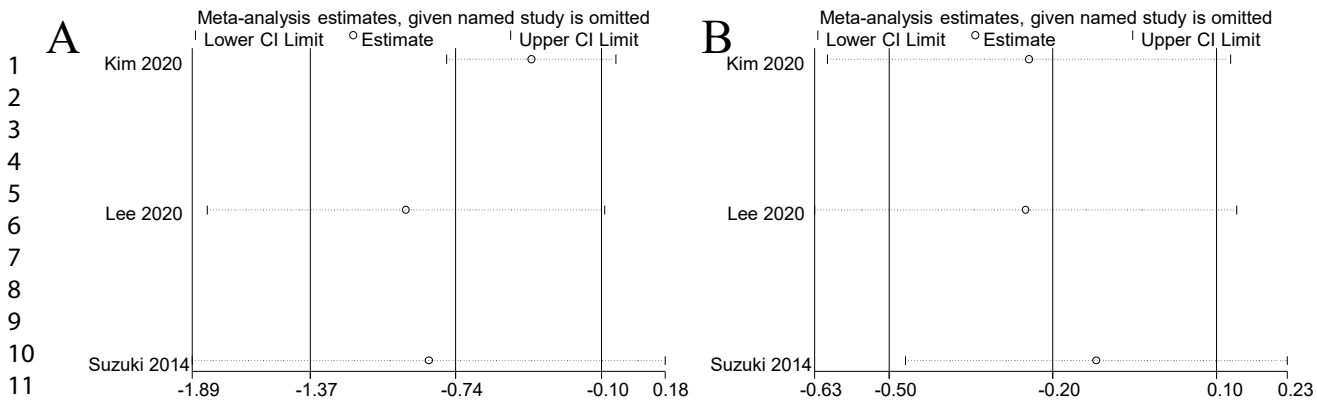


Figure S8: Sensitivity analysis of VSC subgroups in short-term (<=4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.

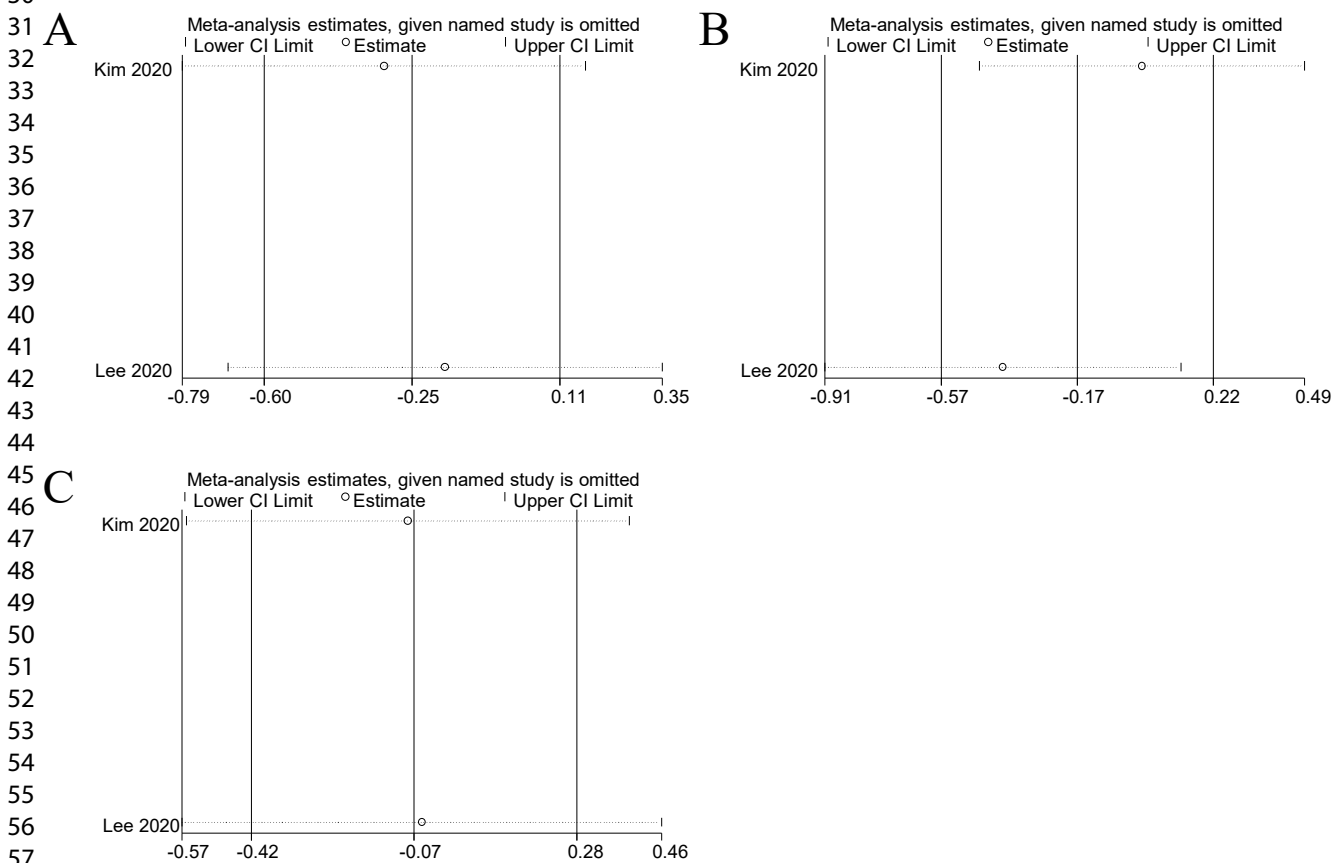


Figure S9: Sensitivity analysis of VSC subgroups in long-term (>4 weeks): (A) H₂S; (B) CH₃S; (C) C₂H₆S.



PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2,3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	3
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	4
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	4
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	4
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	4
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	5
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	5



PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	5
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	5
Study characteristics	17	Cite each included study and present its characteristics.	5
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	5
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	6
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	6
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	6
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	6
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	6
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	6
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	7
	23b	Discuss any limitations of the evidence included in the review.	7,8
	23c	Discuss any limitations of the review processes used.	7,8
	23d	Discuss implications of the results for practice, policy, and future research.	8
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	3
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	3
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	2
Competing interests	26	Declare any competing interests of review authors.	2
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	4

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71

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