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Effectiveness of probiotics in managing oral halitosis: a systematic review

Η αποτελεσματικότητα των προβιοτικών στη διαχείριση της κακοσμίας του στόματος : Συστηματική Ανασκόπηση

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A. Abstract/Περίληψη

Background & Aim Halitosis, or oral malodor, is common and can affect personal and professional relationships. It primarily originates from volatile sulfur compounds (VSC) produced by bacteria in the oral cavity. Traditional treatments, such as mouthwashes, often provide limited success, leading to an increased interest in probiotics as a biological alternative. This systematic review aims to investigate the effectiveness of probiotics as mono-therapy on halitosis by mainly measuring VSC levels and organoleptic (OLT) scores as primary outcomes. **Methods** A systematic search of MEDLINE - PubMed and other databases was conducted for randomized controlled trials (RCTs) involving systemically and periodontally healthy subjects and using placebo as a comparator. Data synthesis followed PRISMA guidelines. **Results** Six studies with 360 participants met the criteria. Different tools, including halimeters and/or Oral Chroma, assessed halitosis. Five studies reported significant reductions in VSC levels, and three showed improved OLT scores with probiotics. Microbiological evaluations in four studies detected probiotic strains post-treatment, with three showing significant changes in oral microbiota. No significant adverse effects were reported. **Conclusion** Probiotics appear effective in reducing halitosis, improving VSC levels, OLT scores, and altering oral microbiota. However, study design variations limit definitive conclusions. Larger, well-designed studies are needed to confirm probiotics' role in managing halitosis.

Keywords: Halitosis, Probiotics, Systematic reviews and evidence-based medicine, VSC, OLT

Εισαγωγή & Στόχοι Η κακοσμία του στόματος, ή δυσοσμία του στόματος, είναι συχνή και μπορεί να επηρεάσει τις προσωπικές και επαγγελματικές σχέσεις. Προέρχεται κυρίως από πτητικές θειούχες ενώσεις (VSC) που παράγονται από βακτήρια στη στοματική κοιλότητα. Οι παραδοσιακές θεραπείες, όπως τα στοματικά διαλύματα, παρέχουν συχνά περιορισμένα αποτελέσματα, οδηγώντας σε αυξημένο ενδιαφέρον για τα προβιοτικά ως βιολογική εναλλακτική λύση. Η παρούσα συστηματική ανασκόπηση έχει ως σκοπό τη διερεύνηση της αποτελεσματικότητας των προβιοτικών ως μονοθεραπεία στην κακοσμία, μετρώντας τα επίπεδα VSC και τους οργανοληπτικούς δείκτες (OLT) ως πρωτεύοντα αποτελέσματα. **Μέθοδοι** Διεξήχθη συστηματική αναζήτηση σε βάσεις δεδομένων, όπως το MEDLINE-PubMed, για τυχαιοποιημένες κλινικές μελέτες (RCTs) που περιλάμβαναν συστηματικά και περιοδοντικά υγιή άτομα και χρησιμοποιούσαν εικονικό φάρμακο (placebo) ως συγκριτικό μέτρο. Η σύνθεση των δεδομένων ακολούθησε τις κατευθυντήριες οδηγίες PRISMA. **Αποτελέσματα** Έξι μελέτες με 360 συμμετέχοντες πληρούσαν τα κριτήρια. Διάφορα εργαλεία, όπως αλομετρητές και/ή συσκευές Oral Chroma, χρησιμοποιήθηκαν για την αξιολόγηση της κακοσμίας. Πέντε μελέτες ανέφεραν σημαντικές μειώσεις στα επίπεδα VSC, και τρεις έδειξαν βελτίωση στους δείκτες OLT με τα προβιοτικά. Η μικροβιολογική αξιολόγηση σε τέσσερις μελέτες εντόπισε προβιοτικά στελέχη μετά τη θεραπεία, ενώ τρεις από αυτές έδειξαν σημαντικές αλλαγές στη μικροχλωρίδα του στόματος. Δεν αναφέρθηκαν σημαντικές παρενέργειες. **Συμπέρασμα** Τα προβιοτικά φαίνεται να είναι αποτελεσματικά στη μείωση της κακοσμίας, βελτιώνοντας τα επίπεδα VSC, τους δείκτες OLT και την αλλαγή της στοματικής μικροχλωρίδας. Ωστόσο, οι διαφορές στο σχεδιασμό των μελετών περιορίζουν τα οριστικά συμπεράσματα. Απαιτούνται μεγαλύτερες, καλά σχεδιασμένες μελέτες για να επιβεβαιωθεί ο ρόλος των προβιοτικών στη διαχείριση της κακοσμίας του στόματος.

Λέξεις-κλειδιά: Κακοσμία του στόματος, Προβιοτικά, Συστηματικές ανασκοπήσεις και ιατρική βασισμένη στην τεκμηρίωση, VSC, OLT

B. Introduction

Halitosis, or bad breath, is a condition characterized by an unpleasant odor originating from the mouth. It significantly impacts social and professional interactions and is among the main complaints addressed in dental practice. According to estimates, the prevalence of halitosis in the general population is 31.8% worldwide, with regional variations and socioeconomic factors influencing prevalence rates that range from 2.4% to 55% in particular populations [1].

The primary origin of halitosis is intraoral, caused by volatile sulfur compounds (VSCs) such as hydrogen sulphide (H_2S), methyl sulphide (CH3SH, or methyl mercaptan), and dimethyl sulphide [(CH3)2 S]. These compounds are by-products of bacterial metabolism involving sulfur-containing substrates in anaerobic oral environments [2,3]. Contributing factors include inadequate oral hygiene, tongue coating, periodontal disease and lifestyle habits such as smoking and alcohol [2]. Tongue coating plays a critical role that contributes to VSC production [2,3].

Conventional management therapies for halitosis include mechanical debridement using tongue scrapers and chemical agents like chlorhexidine [2]. Regardless of the active components, the impact of mouth rinses on oral malodor was examined in two previously published systematic reviews. While conducting limited research on other over-the-counter treatments, both discovered data supporting the fairly positive effects of mouth rinses containing CHX, Zn, and CPC. [4,5]

Due to these challenges, probiotics are a promising alternative. Characterized as living microorganisms that boost the host's health, probiotics can control halitosis by producing antimicrobial compounds, modifying the immune system, and competitively inhibiting harmful bacteria [6]. Additionally, they could be used as an adjuvant therapy to treat dental cavities and periodontal illnesses [6,7].

Certain probiotic strains have shown promise in clinical investigations, such as *Streptococcus salivarius K12* and *Weissella cibaria*, in reducing VSC levels and improving oral health. Despite encouraging results, limitations in study design, small sample sizes, and heterogeneity in probiotic regimens have hindered definitive conclusions [8,9].

The purpose of this systematic study is to assess how well probiotics work to lessen halitosis, comparing them against placebo treatments, and contributing to the broader understanding of their role in dental care.

Following the formulation of the focused question, the review was carried out in compliance with the guidelines of the Preferred Reporting Items for Systematic review (PRISMA) [10].

C. Materials and Methods

Registration

A larger protocol that was created, registered, carried out, and reported in compliance with pertinent methodological guidelines served as the foundation for the current review (OSF Registration DOI https://doi.org/10.17605/OSF.IO/H5UGZ). The current study did not require ethical approval because it is a systematic review.

Focused question

Using the established Population, Intervention, Comparison, Outcome, and Studies (PICOS) framework, the following inquiry was created: What effect does giving probiotics in any form as a monotherapy have on intra-oral malodor in people who are systemically and periodontally healthy, as determined by volatile sulfur compound (VSC) levels and/or organoleptic (OLT) ratings in randomized controlled clinical trials?

Study design

The aim of this review was to evaluate if probiotics are effective compared to a placebo in managing intra-oral halitosis. Therefore, randomized controlled trials were identified as the most suitable research design. Only randomized controlled trials that examined the administration of probiotic strains for managing halitosis of intra-oral origin were included in this review compared to a placebo group.

Inclusion criteria

(a) studies in humans; (b) systematically healthy patients, with no active periodontitis; (c) specified probiotic substance in any form as the only intervention; (d) placebo as a comparator; (e) use of organoleptic scores and/or volatile sulfur compound levels as primary endpoints, to measure the studies' data; (f) sample size >20 patients; (g) randomized clinical trials

Exclusion criteria

(a) animal or experimental studies; (b) no control group; (c) unsupported opinions of experts; (d) editor's choices; (e) replies to authors/editors; (f) interviews; (g) commentaries; (h) book or conference abstracts; (i) summaries; (j) cross-sectional surveys; (k) case series without control; (l) case reports or reports of cases; (m) case-control observational studies; (n) cohort studies; (o)

prospective controlled clinical trials; (p) retrospective clinical trials; (q) narrative reviews; (r) systematic reviews; (s) meta-analyses; (t) studies in a foreign language (non-English); (u) studies with no reported results; (v) in vitro studies; (w) patients with active periodontitis; (x) studies with small sample sizes (<20); (y) studies with multiple interventions; (z) use of chlorhexidine, inulin, tongue scraping, or other disinfection protocols as adjuncts to probiotics or vice versa; (aa) studies without a placebo as comparator; (ab) studies that do not use Organoleptic scores or VSC levels to measure data; (ac) studies without specified probiotics (e.g., probiotic solution or probiotic yogurt).

Primary and secondary outcomes

Primary outcomes were organoleptic scores and/or Volatile Sulphur Compound levels. Secondary outcomes explore associated factors like microbial composition, tongue coating or periodontal parameters.

Database search strategy

(1) MEDLINE (through PubMed); (2) Scopus; (3) Science Direct; (4) Google Scholar; (5) Livivo; (6) Clinicaltrials.gov (National Library of Medicine); (7) Ovid; (8) Grey Literature and (9) Meta Register of Controlled Trials (WHO) were the electronic databases that were searched. In order to find pertinent literature up to and including September 2024, a thorough search strategy was created for each database based on the one used for MEDLINE. This technique is explained in ***Table 1.***

Table 1 Search strategy for identifying papers that investigated the use of probiotics in the management of halitosis

Database	Search strategy used	Limits
Medline- PubMed	(halitosis OR malodor OR malodour OR bad breath OR fetor oris) AND probiotics	No
Scopus	(TITLE – ABS - KEY ((halitosis OR malodor OR "bad breath" OR "fetor oris")) AND TITLE-ABS-KEY (probiotics))	Title, abstract, keywords:
Science Direct	Title, abstract, keywords: [(halitosis OR malodor OR "bad breath" OR "fetor oris")] AND probiotics	Title, abstract, keywords:
Google Scholar	allintitle: halitosis probiotics OR malodor OR "bad breath" OR "fetor oris"	Title
Livivo	TI=(halitosis OR malodor OR malodour OR bad breath OR fetor oris) AND TI=probiotics	Title
Clinical Trials gov	condition or disease:halitosis, other terms: probiotics	No
Ovid	(AllFields: (halitosis OR malodor OR malodour OR "bad breath" OR "fetor oris")AND (probiotics))	All
greylit.org	halitosis and probiotics	No
meta register of controlled trials (https://trialsearch.who.int/)	halitosis and probiotics	No

Search strategy and data extraction

The study selection process will involve two independent reviewers (G.P and C.N) doing the evaluation in different stages. Each reviewer starts by examining both titles and abstracts of each study to see which ones would fit the inclusion requirements. Potentially relevant papers will therefore be subjected to a second more detailed and thorough screening of the full text for the purpose of determining eligibility on the basis of the same criteria. Excluded are the studies which, in an attempt to address the inclusion criteria at any stage, fail to actually satisfy the criteria addressed. In cases where the reviewers differ in their judgment with respect to inclusion of certain studies they will be assisted by a third author (K.P) to resolve the disagreement. To collect data, two reviewers (G.P and C.N) will obtain the relevant information from all included studies at a structured table format. Key data fields will include, study characteristics such the principal features of study design, number of participants, inclusion criteria, evaluation parameters and country of origin/university of research. Other characteristics will include details such as the intervention type, type of the probiotic strain used, primary outcome measures and outcome scores, duration of follow-up period, outcome of the clinical trial and the availability to access of these publications.

Risk of bias

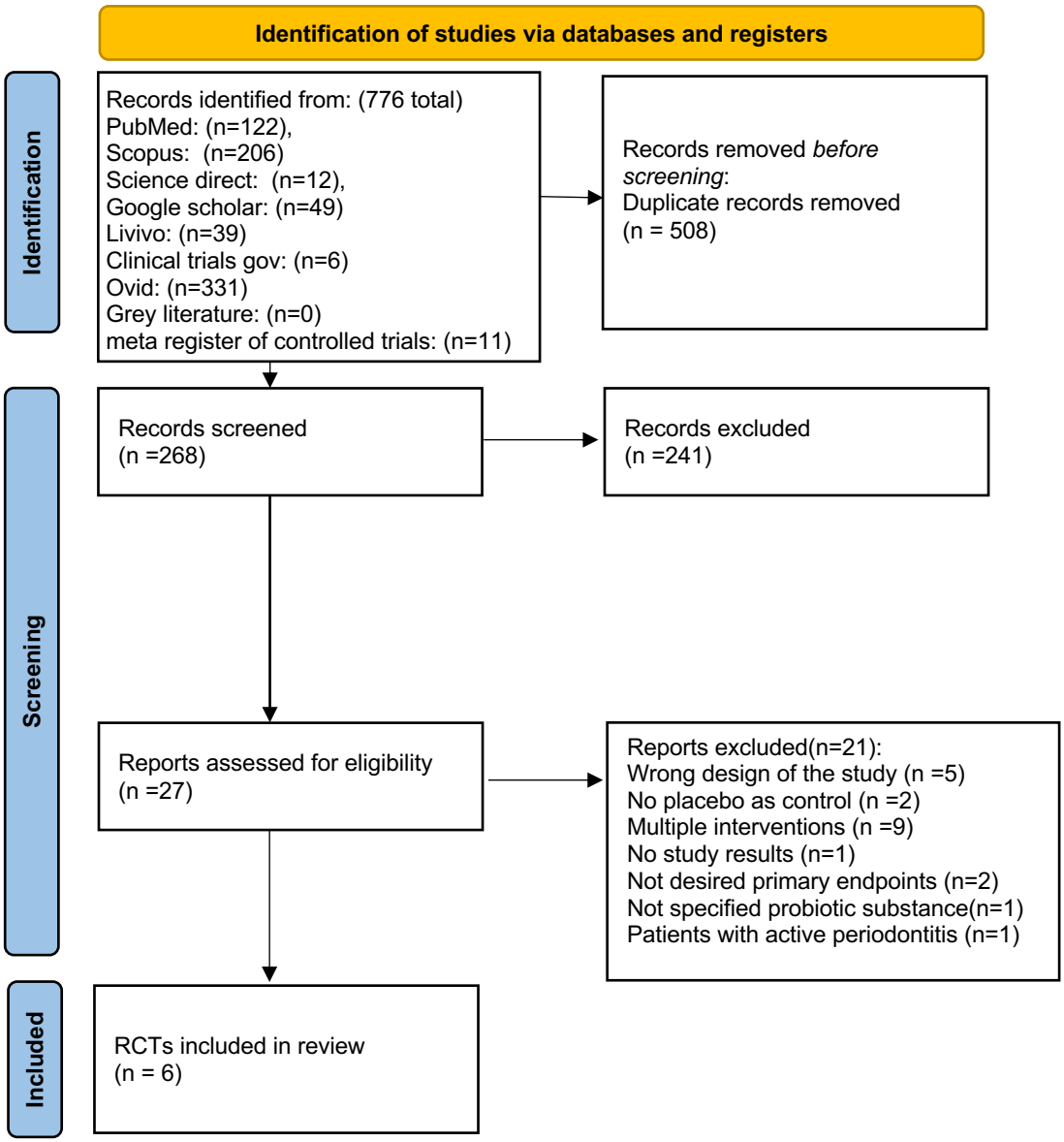
G.P. and C.N. will independently and in duplicate assess the risk of bias in all RCTs using the Cochrane Risk of Bias Tool for Randomized Clinical Trials (RoB 2), which was most recently updated in March 2011 [11]. Bias resulting from the randomization procedure, bias resulting from variations from the intended interventions, bias resulting from missing outcome data, bias in outcome assessment, and bias in the selection of the reported result are among the five areas. Studies will be qualified into three groups based on their risk of receiving bias. If all key domains of the study will have low risk, the bias is not expected to greatly affect the results. Studies will be awarded "some concerns" risk where one or more key domains are assessed as having some concerns, thus this will enhance uncertainty on the findings of the study. Studies will be classified as high risk if one or more important areas are rated as high risk, which suggests bias may lower trust in the dependability of the findings. When necessary, disagreements between investigators will be settled following consultation with a third author (S.D.).

D. RESULTS

Selection process

A thorough database search turned up 776 publications. (***Fig. 1***) [12]. 241 of the 268 papers that remained after duplicates were removed were disqualified following screening of the titles and abstracts. The whole text of 27 articles was evaluated to determine their eligibility. The following were the reasons why the study was excluded from the final review: (a) non-RCT/Wrong study design (n = 5)[13-17]; (b) No placebo as control (n = 2)[18,19]; (c)Multiple interventions (n = 9)[20-28]; (d) studies with no results (n = 1)[29]; (e) Not desired primary endpoints (n=2)[30,31]; (f) Not specified probiotic substance(n=1)[32] and (g)patients with active periodontitis (n=1)[33]. The remaining six studies [34-39] fulfilled all of the inclusion criteria.

Fig.1 Prisma Flow diagram



Description of the Study Population

The features of each population are included in the analysis provided in the ***Table 2***. In respect to periodontal status, all studies included periodontally healthy individuals, while only three of them [36,38,39] provided supplementary information on other indices such as Probing Depth (PD), Gingival Index(GI), Plaque Index (PI), Calculus Index (CI) and Bleeding on Probing (BOP)/Bleeding Index (BI) scores. No data on periodontium were disclosed in three studies [34, 35,37]. All participants, however, had no signs of active periodontitis in the time of inclusion into each study either because they were healthy in advance or because they had undergone pre-treatment to make them free of periodontitis.

Active caries status was reported in three studies [34, 38,39] which included only caries-free individuals. No other studies reported the prevalence of caries.

Being a non-smoker was demanded in two of the studies [38,39], but other studies did not mention smoking behavior among participants other than a smoking ban implemented in Han et al. 2020.

Study Design and Evaluation Period

All six clinical trials used a randomized, placebo-controlled protocol (RCTs). Among them, Keller et al. 2012 employed crossover design, while the others used parallel-group designs. Evaluation periods varied from 14 days [34] to four weeks [36,38] and up to eight weeks [35,37]. Follow-up assessments beyond the intervention period were conducted in three studies[36,38,39] to evaluate sustained effects.

Intervention Treatment and Procedures

Administered means are: a) lozenges [36,38]; b) tablets [35,37]; c) chewing gum [34] and powder [39].

Regarding probiotics, two studies used *W. cibaria CMU [35,37]*, two studies used *S. salivarius* [36,38], one used a combination of *L. reuteri DSM 17938* and *L. reuteri ATCC PTA 5289* [34] and one a combination of *L. Plantarum CCFM1214* and *L. Salivarius CCFM1215* [39].

When we talk about timing, it was stated directly in four studies that the probiotics would be consumed after normal oral hygiene/brushing [35,36,37,38]. Keller et al. 2012 and Ding et al. 2024 didn't mention any means of oral hygiene exactly before the probiotics' intake. Also, in all surveys, apart from two [34,39], it was encouraged that all patients continue their normal hygiene routine and they were prohibited to use any supplementary hygiene means such as mouthwashes or take any other probiotics unrelated to the study.

Table 2 Characteristics of included studies

Study	Country	Study Type	Age range (mean), sex	Number of participants	Pre-treatment	Definition of halitosis	Administration vehicle
Keller et al.(2012)	Denmark	Double-blind, placebo controlled, RCT	19-25(22.0)	28[a]/25[b]	None	OLT>1	Gum
Han et al.(2023)	Korea	Double-blind, placebo controlled, RCT	20-70(51.7) 28M,63F	100[a]/91[b]	None	VSC>1.5ng/ 10ml	Tablet
Benic et al. (2019)	New Zealand	Triple-blind, placebo controlled, RCT	10-30(14.9) 23M, 41F	64[a]/64[b]	None	Not stated	Lozenge
Lee et al. (2020)	Korea	Double-blind, placebo controlled, RCT	>20(23.5) 43M, 25F	92[a]/68[b]	Dental scaling and root planning	VSC>1.5ng/ 10ml	Tablet
He et al. (2020)	China	Double-blind, placebo controlled, RCT	23-44(30.04) 8M, 20F	33[a]/28[b]	Periodontal treatment	OLT score ≥ 2 VSC ≥ 1.5ppb	Lozenge
Ding et al. (2024)	China	Double-blind, placebo controlled, RCT	Age range not stated (22.64) 15M, 18F	43[a]/33[b]	None	VSC ≥ 200ppb	Powder

[a] : Number of participants during the randomization process

[b] : Number of participants whose data were analyzed

Table 2 Characteristics of included studies (continued)

Study	Frequency/ Intervention period	Administration method	Probiotics	Outcomes of interest	Follow-up
Keller et al.(2012)	Twice daily for 14 days	1 hour after food intake, twice daily for 10 minutes	*L. reuteri DSM 17938* (1×10^8 CFU) and *L. reuteri ATCC PTA 5289* (1×10^8 CFU)	VSC, OLT	Baseline and 14^{th} day
Han et al.(2023)	Once daily for 8 weeks	One tablet daily at night before bedtime, after oral hygiene.	1.0×10^8 colony forming units (CFU)	VSC and Bad Breath improvement(BBI)	baseline, 4 weeks, 8 weeks
Benic et al. (2019)	2 lozenges daily for 1 month	After brushing your teeth in the morning and at night, take two lozenges per day.	3.6×10^9 *S. salivarius* colony forming units (CFUs)	Plaque Index (PI), Gingival Index (GI), VSC, and plaque samples	Baseline and 1-month intervention and 3 months' intervention free
Lee et al. (2020)	Once daily for 8 weeks	After brushing your teeth each night before bed, melt one tablet and suck it into your mouth.	$1.0 \cdot 10^8$ colony forming units (CFU)/g of *W. cibaria CMU*	OLT scores, VSC level and BBI	Baseline, on 4 weeks and on 8 weeks
He et al. (2020)	Twice daily for 30 days	Twice a day, after brushing your teeth in the morning and evening, swallow a tablet.	1×10^9 colony forming units (CFU) of *S. salivarius K12*	OLT scores and VSC levels, tongue coating (Ta), thickness of tongue coating (Tt), plaque index (PLI), PD, BI, and BoP%.	Baseline and on the 1^{st}, 7^{th}, and 14^{th} day after the 30-day intervention process
Ding et al. (2024)	Three times daily for 28 days	Melt the powder in your mouth with your saliva, then wait a minute before swallowing.	*L. Plantarum CCFM1214* and *L. Salivarius CCFM1215* $2X10^9$ CFU	VSC SCORES GI, GBI, PLI, CI	Baseline, 7^{th}, 14^{th} , 28^{th} and 35^{th} day

Methodological quality of included studies

One study (Ding et al. 2024) had a high risk of bias, whereas another (Benic et al. 2019) had a low risk. There were some issues with the other four studies. The risk of bias assigned to each domain, as determined by the Cochrane risk-of-bias tool 2.0 [40,41], is shown in ***Table 3*** and is also mentioned below:

Risk of bias during randomisation process: With the exception of one study [Ding et al. 2024], where no details regarding the randomization procedure were given, all of the studies had low bias, indicating that the allocation sequence was sufficiently hidden and random. Baseline imbalances were not disclosed until participants were enrolled and assigned to their interventions. However, this survey was given a "some concerns" bias at this domain because no baseline imbalances indicate a significant issue.

Risk of bias because of deviations from intended interventions: Every study, with very few exceptions, describes a double-blind intervention process. In order to account for missing outcome data, such as patient dropouts during the intervention period, even if there were none, one study examined the impact of assignment to intervention using an intention-to-treat approach [Benic et al. 2019]. It has a low risk of bias because it examined every patient that was allocated. Five research, however, suggest a per protocol analysis. Four of these trials had extremely few patient dropouts in each group, thus there was little chance that the total results would be significantly impacted and there was some concern about bias. One [Ding et al.2024] shows a substantial bias since there were significant dropouts in the control group that were out of proportion to the probiotic group. Generally, the studies did not deviate from the original minumum number of participants required in both control and probiotic groups in order to achieve the desired survey power.

Risk of bias from missing outcome data: Data for all or almost all recruited patients were available in four studies. Two patients were eliminated from one research [Lee et al. 2020] for "abnormal data" without providing a reason. This study was deemed to have some concerns since the percentages of missing outcome data were the same for each group. In one study [Ding et al. 2024], the proportions of the outcome data are not equal so the study was assessed as having high bias.

Risk of bias during the measurement of outcome: In all studies, the method of measuring the outcomes was appropriate and consistent between groups. Additionally, the outcome assessors were either unaware of the intervention received by participants, or the assessment of the outcome could not have been influenced by knowledge of the intervention received. This ensures minimal bias in outcome measurement.

Risk of bias in selection of reported result: All studies had low risk. This is due to the fact that the data were analyzed in compliance with a predetermined plan that was completed prior to the availability of unblinded outcome data for analysis. Furthermore, based on the results, it seems improbable that the outcome being evaluated was chosen from among several eligible outcome measures within the outcome domain or from among several suitable data analyses.

Table 3 Risk of bias of included randomised controlled clinical trials

AUTHOR (YEAR)	RANDOMISATION	DEVIATIONS FROM INTENDED INTERVENTIONS	MISSING OUTCOME DATA	MEASUREMENT OF THE OUTCOME	SELECTION OF THE REPORTED RESULT	OVERALL RISK OF BIAS
KELLER ET AL. (2012)	Low	Some Concerns	Low	Low	Low	Some Concerns
HAN ET AL. (2023)	Low	Some Concerns	Some Concerns	Low	Low	Some Concerns
BENIC ET AL. (2019)	Low	Low	Low	Low	Low	Low
LEE ET AL. (2020)	Low	Some Concerns	Some Concerns	Low	Low	Some Concerns
HE ET AL. (2020)	Low	Some Concerns	Low	Low	Low	Some Concerns
DING ET AL. (2024)	Some Concerns	High	High	Low	Low	High

Synthesis of the results

Effectiveness of Probiotics

The participants of the studies, were advised to abstain from eating and/or drinking and/or brushing of teeth before the assessments. For example, He et al. (2020) included two hours of fasting. However, Lee et al. (2020), He et al. (2020) and Keller et al. (2012) did not wish that the patients follow any oral hygiene the morning of the measurements. Finally, Ding et al. 2024 prohibited the use of irritants such as garlic for 24h while Benic et al. (2019) had no information regarding fasting duration. The results are summarized in ***Table 4***:

Table 4 Methods of assessing halitosis parameters across studies

AUTHOR (YEAR)	INSTRUCTIONS PRIOR TO HALITOSIS ASSESSMENT	EXAMINERS	SCORING SYSTEM OF OLT SCORES AND/OR VSC LEVELS	METHOD FOR OBTAINING SAMPLE FOR ASSESSMENT
KELLER ET AL.(2012)	Avoiding hot foods the night before the exam and refraining from eating, drinking, and maintaining good dental hygiene on the morning of the test	Two aligned organoleptic judges	OLT: Glass tube method 0-5 scoring (Greenman scoring system) o: No odor 1: questionable odor 2: slight malodor 3:moderate malodor 4: strong malodor 5: extremely strong malodor VSC: Halimeter	After initial breath measurements, To produce a standardized malodor, the participants were told to rinse for one minute with 10 milliliters of L-cysteine suspension. Ten minutes after rinsing, the Halimeter was used to measure the malodor.
HAN ET AL.(2023)	Until the morning of the measurement, refrain from eating anything after brushing your teeth in the evening.	One aligned examiner	VSC: Oral Chroma	Five minutes prior to measurements, refrain from speaking and keep your lips shut for thirty seconds while holding a gastight syringe.
BENIC ET AL. (2019)	Not reported	One aligned examiner	VSC: Halimeter	A straw attached to the Halimeter was inserted into a slightly open mouth without coming into contact with the tongue, teeth, or other tissues. The patients were told to breathe via their noses.
LEE ET AL. (2020)	The morning of every appointment, no food or drink, no dental hygiene, and no food that can aggravate bad breath the night before	One aligned trained examiner	OLT: 0-5(Greeenman scoring system) o: No odor 1: questionable odor 2: slight malodor 3: moderate malodor 4: strong malodor 5: extremely strong malodor VSC: Oral Chroma	OLT: Quickly exhale through your lips, 10 cm away from the examiner's nose. VSC: Keep your mouth shut for 30 seconds while holding a gastight syringe in your mouth, and refrain from talking five minutes prior to measurements.
HE ET AL. (2020)	No onions, leeks, garlic and alcohol 24h before the assessment. No eating, drinking, chewing gums or oral hygiene 2h before the assessment	One aligned examiner	OLT: 0-5 o: None 1: barely noticeable 2: slight but clearly noticeable 3: moderate 4: strong offensive 5: extremely foul VSC: Halimeter	OLT : Close mouth for 1 min, exhale slowly form mouth to clinician’s face at 10 cm distance
DING ET AL. 2024)	No irritant such as garlic or shallots 24h before the assessment	Two aligned examiners	VSC: Halimeter	Not reported

All studies used a Halimeter® or Oral Chroma™ to measure VSC levels. OLT scores were only reported in three studies. Keller et al (2012) recorded substantial improvement in organoleptic test scores, however VSC levels did not change. Moreover, Lee et al. (2020) finds that changes in both OLT and VSC levels are important at week 4; however, the findings don't support the positive effect of the probiotics if we keep in mind the measurements on week 8. Han et al. (2023) shows a significant improvement regarding VSC levels and the concentration of H_2S and CH_3SH especially at week 8 of the treatment. In Benic et al. (2019), the decrease in levels of VSC was maintained over a period of 3 months showing the sustained effects of the probiotic. Similarly, He at al. (2020) discovered that both OLT scores and VSC levels had decreased in the two groups if compared to their respective baseline ones the first day following a 30-day intervention period, with sustained effects until the measurements on the 14^{th} day post-intervention, despite a tendency of recurrence . Likewise, Ding et al. 2024 showed a significant decrease in VSCs after two weeks of intervention and sustained effects one week after the 28-day intervention period (35th day).

Assessment of other halitosis parameter

Two surveys [35,37] explored another primary endpoint parameter to measure halitosis: the Bad Breath Improvement (BBI) score [42]. Only at week eight of the intervention did both find significant differences between the test and control groups; at week four, they did not. The BBI scores tended to decrease after 8 weeks of intervention.

Assessment of secondary endpoints

Three studies [36,38,39] investigated other intra-oral parameters as their secondary endpoints. He et al. 2020 worked with Tongue Coating Scores (TCS) and various periodontal indices such as Plaque Index (PI), Probing Depth (PD), Bleeding Index (BI) and Bleeding on Probing (POD%), unable to find any significant differences between groups despite their improvement in the probiotics group. Likewise, Benic et al. 2019 did not find significant differences between groups based on their PI and Gingival Index (GI) scores, highlighting stable periodontal health during and post intervention. Finally, Ding et al. 2024 found a significant difference only on Calculus Index (CI) and GI scores after 28 days of intervention but not on PI and Gingival Bleeding Index (BLI) ones.

Microbiological Outcomes

In four studies, microbiological analysis was performed. According to Han et al. (2023) and Lee et al. (2020), who collected tongue samples, there were notable variations between the groups in the percentage of Weissella Cibaria from baseline to 8 weeks. Ding et al. (2024) employed saliva samples that demonstrated a substantial decrease in F. nucleatum in the probiotic group compared to the control group, as well as a relatively higher abundance of Proteobacteria and lower levels of Fusobacteria. Lastly, Benic et al. (2019) didn't find any promising data regarding the presence of *S. salivarius* between treatment groups and time periods by taking tooth plaque samples. The remaining two studies didn't pursue any bacterial quantitative analysis (Keller et al. 2012; He at al. 2020)

Safety and Tolerability

It has been established that incorporating probiotics into one's daily regimen is very safe. During the trials there were no critical adverse effects noted, and the subjects suffered no pain or discomfort or any adverse reactions while the interventions lasted. The results also indicated that there were high rates of compliance. This means that probiotics were not harmful to the subjects. The fact that no adverse effects were noted allows one to conclude that such probiotics can be safely applied as adjunct to any oral hygiene program. As a result, this evidence proves that probiotics not only are useful but also provide good tolerance in the management of bad breath.

E. Conclusion

This systematic review represents probiotics as a very potential biological alternative in controlling and managing halitosis of intra-oral origin, based on a significant reduction of volatile sulfur compounds, improved organoleptic scores, as well as am alteration of the oral microbiota in the included trials. The current review shows that not only could probiotics improve oral breath, but they were also safe, with good compliance. However, the heterogeneity in the designs of these studies, the probiotic strains used, dosages, and evaluation methods limit definitive conclusions. To establish clinical utility and standardization of protocols, future research needs to be large in scale, with well-designed trials, uniform methodologies, and longer follow-up periods. Despite these limitations, the findings affirm the promising role of probiotics as a supplementary approach in dental hygiene.

F. Discussion

With an emphasis on primary outcomes including decreases in volatile sulfur compound (VSC) levels and enhancements in organoleptic (OLT) scores, this systematic review sought to assess how well probiotics manage oral halitosis. While pointing out drawbacks and potential directions for further study, the results shed light on the potential of probiotics as a therapeutic intervention for this illness.

The results from the included studies suggest that probiotics, particularly strains such as *Streptococcus salivarius K12* and *Weissella cibaria*, show promise in reducing VSC levels and improving OLT scores compared to placebo. The mechanisms likely involve alterations in the oral microbiota, with a shift toward a more favorable microbial composition that reduces the production of malodorous compounds. However, heterogeneity in the reported results, study designs, and probiotic strains limit the ability to draw definitive conclusions. While some studies demonstrated statistically and clinically significant improvements, others reported minimal or no effects, underscoring variability in study quality, intervention protocols, and outcome measures.

Strengths and Implications: One of the few reviews that thoroughly assesses the effectiveness of probiotics in treating oral halitosis, mostly in patients without active periodontitis, is this one. The review reduces the possibility of publication bias and offers a comprehensive summary of the available information by incorporating a thorough search of numerous databases, trial registries, and grey literature

sources. The results provide credence to probiotics' potential use as an adjuvant, non-invasive treatment for oral halitosis. Given the increasing consumer preference for natural and non-pharmaceutical interventions, these results have clinical relevance and public health implications. Probiotics could serve as an accessible option for individuals seeking to manage halitosis without reliance on chemical-based mouthwashes or antibiotics.

Limitations: This review has a number of shortcomings in spite of its advantages. First, only a small number of studies were considered, and many of them had short follow-up periods and small sample sizes. This limits the capacity to evaluate the long-term effectiveness of probiotics in treating halitosis and decreases the data' generalizability. Second, heterogeneity in study design, probiotic strains, dosage regimens, and outcome measures complicates direct comparisons across studies. For example, the lack of standardization in how VSC levels and OLT scores were measured introduces variability that could influence results. Third, one study had a high risk of bias, especially when it came to missing outcome data and variations from the planned interventions, which calls into question the overall findings of this study [39]. As a result, these factors may overestimate the reported effects of probiotics.

Comparison with Existing Literature: The review's findings are consistent with past studies that highlighted the potential benefits of probiotics for oral health, including periodontal treatment and cavity prevention. However, the evidence for benefits specific to halitosis is currently sparse and inconsistent. Probiotics appear to improve the oral microbiota, but further research is needed to identify their precise mechanisms of action and identify the most effective strains and dosages.

Future Directions: High-quality randomized controlled trials with bigger sample numbers and standardized outcome measures ought to be given priority in future studies. To evaluate the long-term benefits of probiotics and ascertain their function in halting the return of halitosis, longer-term research is especially required. Additionally, comparative studies investigating different strains, dosages, and delivery methods could help identify the most effective probiotic formulations for clinical use. Finally, more robust reporting of adverse effects is essential to evaluate the safety of probiotics in the long run. While probiotics are generally considered safe, detailed documentation of side effects in diverse populations will enhance their clinical applicability.

Compliance with Ethical Standards

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

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