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ORIGINAL ARTICLE

Effect of chewing gums containing the probiotic bacterium *Lactobacillus reuteri* on oral malodour

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Abstract

Objective. To evaluate the effect of chewing gums containing probiotic bacteria on oral malodour. The null hypothesis was that no difference would be displayed compared with placebo gums. ***Materials and methods.*** Twenty-five healthy young adults with self-reported malodorous morning breath completed this randomized double-blind placebo-controlled cross-over trial. The design included run-in and wash-out periods interspersed by two intervention periods of 14 days each. The subjects were instructed to chew one gum in the morning and one in the evening containing either two strains of probiotic lactobacilli (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289) or placebo. The outcome measures were (i) organoleptic scores (0–5) by a certified test panel, (ii) concentration of volatile sulphur compounds (VSC) measured with a Halimeter and (iii) concentration of VSC after a cysteine rinse. Registrations were made at baseline and after each intervention period. Differences between the groups were assessed by non-parametric paired statistics and chi-square test. ***Results.*** The median organoleptic score was similar (score 2) in both groups at baseline. After 14 days of treatment, the organoleptic scores were significantly lower in the probiotic group compared with the placebo group ($p < 0.05$). Assessments of the VSC levels displayed no significant differences between the groups, either before or after rinsing with L-cysteine. No adverse effects were registered. ***Conclusion.*** The results demonstrated that probiotic chewing gums may have some beneficial effect on oral malodour assessed by organoleptic scores. The results indicate that the probiotic gum may affect bacteria that produce malodourous compounds other than VSCs.

Key Words: *halitosis, organoleptic scores, probiotics, VSC*

Introduction

Oral malodour is an unpleasant, but common condition that may affect up to 30% of the population [1]. The majority of all cases are caused by malodourous compounds that come from the oral cavity itself. The malodourous compounds may also be produced by nasal, oro-pharyngeal and pharyngeal infections, as well as by respiratory, gastro-intestinal and metabolic conditions [2,3]. In addition, dietary sources such as spicy food and tobacco also contain compounds that can be perceived as unpleasant or malodourous.

Within the oral cavity, malodourous compounds can be produced by a combination of anaerobic bacteria, food residues, exfoliated epithelial cells and salivary proteins that accumulate in the fissures of the tongue. Thus, a positive relation between malodour and amount of tongue coating has been demonstrated [4–6]. For these relations, the morphology of the tongue with fissures may create an environment that favours the growth of anaerobic bacteria that produce volatile compounds as a result of their metabolic degradation [6,7]. Among oral pathological conditions that can cause oral malodour, chronic periodontitis is considered the most common factor [3,8]. In general, amino acids are the main substrate for the production of oral malodourous compounds. As freshly secreted human saliva contains low levels

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of free amino acids, halitosis occurs as a result of bacterial putrefaction by several anaerobic species found in the oral cavity. This leads to decomposition of proteins, peptides and amino acids and the generation of volatile sulphur compounds (VSC) such as hydrogen sulphide, foul smelling amines (putrescine and cadaverine) and indole and skatole from aromatic amino acids [5,9,10].

The current treatment options of oral malodour focus on reducing the total bacterial numbers by mechanical measures such as brushing, flossing and tongue scraping, antibacterial agents and complex binding of malodourous compounds by zinc [7,11,12]. As these strategies have a relative short-termed effect, it has been postulated that bacteriotherapy in order to replace halitosis-associated species may create a more long-lasting effect [13]. Preliminary reports from studies with bacteriocin-producing *S. salivarius* and *Weissella cibaria* have suggested a reduction of the VSC levels [14,15], but further studies are needed. Therefore, the aim of the present study was to evaluate the effect of chewing gums containing two probiotic lactobacilli strains on oral malodour in a group of young adults. The null hypothesis was that no differences would be obtained when compared with a similar use of placebo chewing gums. A second aim in the preparatory phase was to evaluate four different clinical methods for assessment of oral malodour.

Materials and methods

Subjects

Within the Faculty of Health Sciences in Copenhagen public notices were used to recruit healthy volunteers with self-perceived bad breath on awakening for an initial interview and screening. The inclusion criteria were (i) subjective feeling of bad breath in the mornings, (ii) age between 19–30 and (iii) being a non-smoker. The exclusion criteria were (i) ongoing treatment with antibiotics, (ii) xerostomia, (iii) periodontal disease or rampant decay with excessive open caries lesions or (iv) any known chronic illnesses. After screening, the study group consisted of 30 young adults (20 females and 10 males) within the age range of 19–25 years and with a mean age of 22 years. Prior to participation, all subjects received verbal and written information and signed a consent form. Two subjects were excluded at baseline because the organoleptic score was judged to be less than one. During the course of the study, three subjects dropped out due to relocation (two cases) and lack of adherence to the protocol due to a stressed life situation (one case). Thus, the final results were based on 25 persons. The protocol was approved by the Ethical Committee in the Capital Region of Denmark (H-D-2008-073).

Study design

The prospective study was designed as a double-blind placebo-controlled randomized cross-over trial. After inclusion, subjects were randomly assigned to one of the two study groups by throwing a dice. Group A (n = 16) was given the active chewing gums and group B (n = 12) received the chewing gum without any added bacteria. Registrations were conducted at baseline and after 2 weeks. After a 3-week wash-out period, the baseline registrations were renewed before the second intervention period of 2 weeks.

Intervention

Probiotic and placebo chewing gums had identical taste, size and colour with a base consisting of isomalt, hydrogenated palm oil, sucrose esters of fatty acids, peppermint oil, menthol flavour and sucralose. In addition, the probiotic chewing gum contained *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289—both at the concentration of 1×10^8 CFU/chewing gum. Both gums were produced and provided by BioGaia AB (Lund, Sweden). The participants were instructed to chew the assigned gum, one piece in the morning and one piece in the evening for 10 min at least 1 h after food intake. The chewing gums were packed in identical pots and labelled by a colour code. All clinical registrations were carried out without knowledge of the respective group assignment.

Organoleptic measurements

Prior to experiment, the organoleptic panel, consisting of three certified organoleptic judges (UWE, Bristol, UK), evaluated four different clinical methods for assessment of oral malodour on a group of female volunteers (n = 20) with a clinical diagnosis of oral malodour. The test persons were placed in a chair in a relaxed position and asked to have their mouth closed for 1 min before assessment. In the first method, the subject was asked to lean backwards and to open her mouth to a sub-maximum position (<50 mm) while the organoleptic judge assessed the level of oral malodour at a distance of maximally 5 cm (open mouth method). In the second method, the subject was asked to count to 10 in English while the organoleptic judge assessed the level of oral malodour at a distance of 10 cm (counting method). The third method was similar except that the subject was asked to say 'HA-HA-HA' in a medium loud voice (HA-HA-HA method). In the fourth method, the score was given while the subjects were asked to breathe their expiration air through a 30 cm glass tube from a distance of 5 cm at the end of the tube (glass tube method). For all methods, score 0 was given for no odour; 1 for barely noticeable odour; 2 for slight odour; 3 for moderate odour; 4 for strong odour;

and 5 for extremely strong odour, according to a 0–5 scale and preferably by use of integers only [9]. For each volunteer (V) and method (M), the mean score ($\bar{\chi}_V$), the standard deviation between the three judges (SD_V) and the percentage relative standard deviation ($\%RSD_V$) were calculated. Afterwards the combined mean score and standard deviation for the method (i.e. $\bar{\chi}_M$ and SD_M) was calculated as: $\sum \bar{\chi}_V/n$ and $\sum SD_V/n$. In ascending order the scores were: 0.8 ± 0.5 (open mouth), 1.4 ± 0.5 (counting), 1.5 ± 0.5 (HA-HA-HA) and 2.0 ± 0.6 (glass tube) revealing a significant difference among the methods ($p < 0.001$). In terms of relative standard deviation for each method (i.e. $\%RSD_M$ calculated as $\sum \%RSD_V/n$) the results were 79 ± 47% (open mouth), 43 ± 21% (counting), 42 ± 27% (HA-HA-HA) and 32 ± 26% (glass tube), which was also significantly different among methods ($p < 0.01$). According to these results only the glass tube method produced an acceptable inter-examiner precision, defined here as when two times RSD_M (i.e. $\%RSD_M/100$) of the mean ($\bar{\chi}_M$) was less or equal to the square root of the mean:

$$\text{Glass tube method} = \frac{2\,RSD_M \cdot \bar{\chi}_M}{\sqrt{\bar{\chi}_M}} \leq 1$$

Clinical procedures

The participants were instructed not to eat or drink and to avoid any oral hygiene measures on the morning of the tests and to refrain from eating spicy food on the evening before. Their breath was then assessed by two different methods; (i) by the certified organoleptic panel consisting of a minimum of two judges at all examinations and (ii) by measurements of the concentration of volatile sulphur compounds (VSC) before and after a standard cysteine challenge [16]. Due to high inter-examiner precision the glass tube method was used to determine organoleptic scores in all tests regarding the gums and according to the 0–5 scale for the degree of oral malodour [9]. The score from each organoleptic judge was hidden from the other judge(s) until an average score was calculated. Secondly, the concentration of VSC was measured using a Halimeter (Interscan Instruments, Chatsworth, CA) according to Rosenberg and co-workers [17,18]. After the initial measurements of the breath, the participants were instructed to rinse with 10 ml of L-cysteine suspension for 1 min to create a standardized malodour. The breath was then measured with the Halimeter, 10 min after rinsing with the suspension.

Statistical methods

All data were processed by the IBM SPSS Statistics software (version 19.0, Chicago, IL). Differences between the four organoleptic methods were determined by the Kruskal-Wallis rank-sum test. Differences between the groups were assessed with Chi-square tests (organoleptic scores) and with the non-parametric Wilcoxon signed-rank test (VSC-recordings). A p-value less than 0.05 was considered as statistically significant.

Results

The distribution of the organoleptic scores at baseline and follow-up is presented in Table I. There were no differences between the groups at baseline (median score 2), but after 14 days of intervention, significantly lower organoleptic scores in the probiotic test group were observed ($p < 0.05$). The concentration of VSC in unprovoked and provoked breath is shown in Table II. The VSC levels showed a wide range, both in the provoked and unprovoked state. However, the assessments did not display any significant differences between the groups, neither before nor after the intervention. No adverse effects from chewing the gums were registered.

Discussion

This study was undertaken to investigate the possible influence of bacteriotherapy on oral malodour. Although the volunteers were included on the basis of self-perceived bad breath on awakening, it should be noted that not all subjects displayed objective signs of malodour at the baseline registration. Furthermore, they were young adults and did not suffer from periodontal disease or dry mouth. Thus, the findings from this partly pseudo-halitosis study group may not be representative for elderly or for patients with severe bad breath or halitosis caused by more complex oral conditions.

Measurements of volatile sulphur compounds are commonly used for detection of halitosis, but as oral malodour may comprise agents other than VSCs, this

Table I. Percentage distribution of organoleptic scores (0–5) before and after use of probiotic lozenges containing *L. reuteri* (test) or placebo in young healthy adults (n = 25).

	Organoleptic score				
	0–1	1–2	2–3	3–5	p-value
Test BL	—	36%	32%	32%	
Placebo BL	—	40%	28%	32%	NS
Test FU	28%	12%	32%	28%	
Placebo FU	8%	28%	28%	36%	< 0.05

BL, baseline; FU, follow-up; NS, not statistically significant. p-values were obtained by chi-square test.

Table II. Halimeter recordings before and after use of probiotic chewing gum containing *L. reuteri* (test) or placebo in young healthy adults (n = 25). Values in table denote mean (SD).

	Unprovoked[a]		Provoked[a]	
	BL	FU	BL	FU
Test (VSC ppb)	60 (59)	92 (110)	954 (600)	962 (583)
Placebo (VSC ppb)	81 (81)	76 (71)	1233 (572)	1011 (630)
p	NS	NS	NS	NS

[a]Unprovoked states that the breath was assessed before cysteine rinse and provoked after a cysteine rinse.
BL, baseline; FU, follow-up; VSC, volatile sulphur compounds; NS, not statistically significant differences between the groups (Wilcoxon paired test).

method can fail to assess bad breath caused by non-sulphide components [11]. The rationale of using L-cysteine rinses was to provide a maximum substrate for the VSC-producing bacteria and thereby eliminate possible differences between the groups caused by lack of substrate [16]. The compliance with the study protocol was considered as good based on personal interviews and continuous contact with the participants during the course of the study and no complaints with respect to the protocol were evident. Certified judges were used for the organoleptic measurements and four methods were evaluated and compared prior to testing the gums. By breathing through a glass tube during organoleptic measurements, one third of the subjects had scores of moderate-to-strong oral malodour at baseline and another third had scores of slight malodour. As described above, the glass tube method may produce higher values than the open mouth or counting method. However, we argue that false positive findings of oral malodour, if such exist, are a smaller problem than false negative findings and, thus, the higher precision obtained with the glass tube method was preferred. We made no effort to assess the nature of the malodourous compound, but a general impression among the three judges was that many cases of moderate and strong oral malodour were due to compounds other than VSC.

The novel finding in the present study was that daily use of chewing gums containing probiotic lactobacilli had a slight, but statistically significant, beneficial effect on oral malodour in young adults when evaluated by organoleptic scores. No effect was, however, detected on the levels of VSC, which could be explained by the fact that volatile sulphur compounds measured by the Halimeter account for only 18–48% of the organoleptic score [19]. Our findings were, however, in some contrast to earlier findings after daily intake of lozenges with bacteriocin-producing *S. salivarius* K12 [14] or gargling with clinical isolates of *Weissella cibaria* [15]. The latter were hydrogen-peroxide generating strains from children and the two studies indicate a possible role of broad-spectrum bacteriocins in the inhibition of VSC. The two probiotic *L. reuteri* strains used in this study are also known to produce a specific bacteriocin called reuterin but only in the presence of glycerol [20]. The generally low levels of glycerol in the oral biofilm might be one of the reasons for the negative results of the VSC measurements. Nevertheless, it must be assumed that unaltered levels of VSC, with and without provocation with L-cysteine, suggest that the amount of VSC-producing bacteria was unchanged after exposure to the probiotic *L. reuteri* strains.

With the present and somewhat encouraging findings on oral malodour in mind, it could be of interest to further speculate on the mode of probiotic action. The most widely used strategies in the treatment of halitosis are comprehensive oral hygiene, including tongue scraping and brushing, as well as the use of mouth rinses containing antibacterial agents. Such treatments may only provide a short-termed relief of halitosis since the problem bacteria quickly will repopulate the tongue and the oral biofilm [13]. The use of benign, commensal probiotics could therefore offer a complementary and more long-term treatment strategy to combat bad breath. Probiotic bacteria can grow in saliva and compete for binding sites in the oral biofilm, although a permanent colonization is unlikely in adults [21]. This may affect the local ecology and prevent re-establishment of non-desirable bacterial populations. Furthermore, the possibility of systemic effects through modulation of the immune response cannot be excluded [22]. Whether or not the bacterial composition was altered in the present study remains an open question, but long-term studies are needed to evaluate the microbial profile of oral microbiota, with special emphasis on non-VSC producing bacteria, to further elucidate the research question. It should also be underlined that the effect was not striking from a clinical point of view, nor subjectively or objectively, and the probiotics therapy may at its best only be an adjunct to the current treatment options.

In conclusion, the results demonstrated that daily use of probiotic chewing gum can have a slight beneficial effect on oral malodour assessed by organoleptic scores. The results indicate that the regime may affect bacteria that produce compounds other than the volatile sulphur compounds and thereby contribute to malodour. Thus, probiotic bacteria may have a potential application as an adjunct to oral hygiene improvement and antibacterial rinses for the management of oral malodour.

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References

[1] Hughes FJ, McNab R. Oral malodour–a review. Arch Oral Biol 2008;53(Suppl 1):1–7.
[2] Delanghe G, Ghyselen J, van Steenberghe D, Feenstra L. Multidisciplinary breath-odour clinic. Lancet 1997;350:187.
[3] Quirynen M, Dadamio J, van den Velde S, De Smit M, Dekeyser C, Van TM, et al. Characteristics of 2000 patients who visited a halitosis clinic. J Clin Periodontol 2009;36:970–5.
[4] De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J Am Dent Assoc 1995;126:1384–93.
[5] Scully C, Greenman J. Halitosis (breath odor). Periodontol 2000 2008;48:66–75.
[6] Haraszthy VI, Zambon JJ, Sreenivasan PK, Zambon MM, Gerber D, Rego R, et al. Identification of oral bacterial species associated with halitosis. J Am Dent Assoc 2007;138:1113–20.
[7] Fedorowicz Z, Aljufairi H, Nasser M, Outhouse TL, Pedrazzi V. Mouthrinses for the treatment of halitosis. Cochrane Database Syst Rev 2008;8:CD006701.
[8] Rosenberg M. Bad breath and periodontal disease: how related are they? J Clin Periodontol 2006;33:29–30.
[9] Greenman J, Duffield J, Spencer P, Rosenberg M, Corry D, Saad S, et al. Study on the organoleptic intensity scale for measuring oral malodor. J Dent Res 2004;83:81–5.
[10] Persson S, Edlund MB, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. Oral Microbiol Immunol 1990;5:195–201.
[11] Porter SR, Scully C. Oral malodor (halitosis). BMJ 2006;23: 632–5.
[12] Outhouse TL, Fedorowicz Z, Keenan JV, Al-Alawi R. A Cochrane systematic review finds tongue scrapers have short-term efficacy in controlling halitosis. Gen Dent 2006; 54:352–9.
[13] Burton JP, Chilcott CN, Tagg JR. The rationale and potential for the reduction of oral malodour using Streptococcus salivarius probiotics. Oral Dis 2005;11(Suppl 1):29–31.
[14] Burton JP, Chilcott CN, Moore CJ, Speiser G, Tagg JR. A preliminary study of the effect of probiotic Streptococcus salivarius K12 on oral malodour parameters. J Appl Microbiol 2006;100:754–64.
[15] Kang MS, Kim BG, Chung J, Lee HC, Oh JS. Inhibitory effect of Weissella cibaria isolates on the production of volatile sulphur compounds. J Clin Periodontol 2006;33: 226–32.
[16] Kleinberg I, Codipilly DM. Cysteine challenge testing: a powerful tool for examining oral malodour processes and treatments in vivo. Int Dent J 2002;52(Suppl 3):221–8.
[17] Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. J Dent Res 1991;70:1436–40.
[18] Rosenberg M. Clinical assessment of bad breath: current concepts. J Am Dent Assoc 1996;127:475–82.
[19] Loesche WJ, Kazor C. Microbiology and treatment of halitosis. Periodontol 2000 2002;28:256–79.
[20] Cleusix V, Lacroix C, Vollenweider S, Le Blay G. Glycerol induces reuterin production and decreases Escherichia coli population in an in vitro model of colonic fermentation with immobilized human feces. FEMS Microbiol Ecol 2008;63: 56–64.
[21] Haukioja A. Probiotics and oral health. Eur J Dent 2010;4: 348–55.
[22] Teughels W, Van Essche M, Sliepen I, Quirynen M. Probiotics and oral healthcare. Periodontol 2000 2008;48:111–47.