Orodispersible lozenges containing a combination of Lactobacillus reuteri DSM 17938 and Lactobacillus reuteri ATCC PTA 5289 and normal gum function: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

Turck Turck, Jacqueline Castenmiller, Stefaan de Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J. Mcardle, Androniki Naska, et al.

SCIENTIFIC OPINION

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EFSA Panel on Nutrition, Novel foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Frank Thies, Sophia Tsabouri, Marco Vinceti, Jean-Louis Bresson, Yolanda Sanz and Alfonso Siani

Abstract

Following an application from BioGaia AB submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Sweden, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to orodispersible lozenges containing a combination of *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 and normal gum function. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The Panel considers that orodispersible lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 are sufficiently characterised. Maintenance of normal gum function is a beneficial physiological effect. Out of the two studies from which conclusions could be drawn and that investigated the effect of lozenges containing *L. reuteri* at the proposed conditions of use (i.e. consumption twice daily) on appropriate gingival outcomes (bleeding on probing (PoB) and gingival index (GI)) in subjects with gingivitis, but without periodontitis, one showed a large effect on BoP and other gingival outcomes and one showed no effect. No effect was found in one study with the use of one lozenge daily. The three studies that investigated, at the proposed conditions of use, modified GI (and not BoP or GI) in subjects with gingivitis, but without periodontitis, or were conducted in patients with periodontitis support an effect of lozenges with *L. reuteri* on gum function. Some evidence has been provided for mechanisms by which consumption of lozenges containing *L. reuteri* could improve outcomes of gingivitis in patients with chronic periodontitis but the relevance of such mechanisms for the target population of the claim (i.e. subjects without periodontitis) is unclear. The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of orodispersible lozenges containing a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 and maintenance of normal gum function.

Keywords: *L. reuteri* DSM 17938, *L. reuteri* ATCC PTA 5289, gum function, gingivitis, health claim

Requestor: Competent Authority of Sweden following an application by BioGaia AB

Question number: EFSA-Q-2019-00383

Correspondence: nda@efsa.europa.eu

Panel members: Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, John Kearney, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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Competing interests: A waiver was granted to an expert of the working group, Jean-Louis Bresson. Pursuant to Article 21(6) of the afore-mentioned Decision, the concerned expert was allowed to take part in the discussion and in the drafting phase of the scientific output.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3). According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

1.2. Interpretation of the Terms of Reference

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 and normal gum function.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289, a positive assessment of its safety, nor a decision on whether a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

2. Data and methodologies

2.1. Data

Information provided by the applicant

Food/constituent as stated by the applicant

According to the applicant, the food for which the health claim is made is 'lozenges with two *Lactobacillus reuteri* strains: *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289, approximately equal amount of each strain (total minimum amount of 1×10^8 CFU/lozenge of each bacterial strain)'.

Health relationship as claimed by the applicant

According to the applicant, the health effect is related to 'support normal gum function. Both gingival indexes (GI) and bleeding on probing (BoP) are used as appropriate study outcomes for assessing the claimed effect'.

Mechanism by which the food/constituent could exert the claimed effect as proposed by the applicant

The applicant claims that 'The mechanism of action can be divided into four general areas: viability and biofilm adhesion, anti-inflammatory properties, anti-microbial properties and effects on barrier function'. Both *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 are able to form biofilms and adhere to the oral mucosa. The total viable count and obligate anaerobes of oral bacteria are reduced by administration of *L. reuteri* DSM 17938 *and L. reuteri* ATCC PTA 5289 in adjunct to scaling and root planing (SRP). *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 have been shown clinically to reduce pro-inflammatory cytokines, tumour necrosis factor α (TNF-α) and interleukin-8 (IL-8), in gingival crevicular fluid of otherwise healthy patients with gingivitis, while also reducing other visible signs of inflammation such as BoP. *L. reuteri* is well known for its ability to produce reuterin. Reuterin is an

antimicrobial compound, which induces oxidative stress in cells and can inhibit the growth of both Gram-positive and Gram-negative bacteria. In an infection model based on porcine intestinal cell line IPEC-J2, the pretreatment of the intestinal epithelial cells with *L. reuteri* DSM 17938 reduced the detrimental effect of enterotoxigenic *Escherichia coli* in a dose-dependent manner, as monitored by transepithelial electrical resistance and translocation of Fluorescein isothiocyanate-dextran (FITC-dextran) over the epithelial layer. *L. reuteri* DSM 17938 may have protective effects also on the mucosal barrier of gingiva.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: 'Lozenges containing *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 support normal gum function'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'general European population'. The daily consumption of two lozenges (one lozenge taken twice daily) containing a total minimum amount of 1×10^8 colony forming units (CFU) of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289, approximately equal amounts of each strain is recommended.

Data provided by the applicant

The health claim application on a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 and normal gum function pursuant to Article 13.5 of Regulation 1924/2006, was presented in a common and structured format as outlined in the Scientific and technical guidance for the preparation and presentation of applications for authorisation of health claims.

As outlined in the General guidance for stakeholders on health claim applications, it is the responsibility of the applicant to provide the totality of the available evidence.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of health claim applications is outlined in the EFSA general guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016).

The scientific requirements for health claims related to bone, joints, skin, and oral health are outlined in a specific EFSA guidance (EFSA NDA Panel, 2012).

The data claimed as confidential are: complete 16S ribosomal RNA gene sequence of *L. reuteri* DSM 17938, complete 16S ribosomal RNA gene sequence of *L. reuteri* ATCC PTA 5289, detailed analytical data on the product characteristics and an unpublished study report by Hasturk (2015). EFSA has issued its Decision on Confidentiality on 8/01/2020.

The application does not contain data claimed as proprietary.

3. Assessment

The approach used by the NDA Panel for the evaluation of health claims is explained in the General scientific guidance for stakeholders on health claim applications (EFSA NDA Panel, 2016). In assessing each specific food/health relationship, which forms the basis of a health claim the NDA Panel considers the following key questions:

(i) the food/constituent is defined and characterised;
(ii) the claimed effect is based on the essentiality of a nutrient; OR the claimed effect is defined and is a beneficial physiological effect for the target population and can be measured *in vivo* in humans;
(iii) a cause and effect relationship is established between the consumption of the food/constituent and the claimed effect (for the target group under the proposed conditions of use).

Each of these three questions needs to be assessed by the NDA Panel with a favourable outcome for a claim to be substantiated. In addition, an unfavourable outcome of the assessment of questions (i) and/or (ii) precludes the scientific assessment of question (iii).

3.1. Characterisation of the food/constituent

The food/constituent proposed by the applicant as the subject of the health claim is a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289. Both *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 have been identified at species level by sequence analysis of whole 16S rRNA gene and at strain

level by a strain-specific polymerase chain reaction (PCR) method based on the whole-genome information of the strain ATCC 55730.

L. reuteri DSM 17938 was deposited in the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen; DSMZ) under the reference number DSM 17938. *L. reuteri* ATCC PTA 5289 was deposited in the American Type Culture Collection (ATCC) under the reference number ATCC PTA 5289.

The strain *L. reuteri* DSM 17938 was derived from *L. reuteri* ATCC 55730 and obtained by curation of two plasmids with antibiotic resistance genes (non-GMO) and was originally isolated from human breast milk. *L. reuteri* ATCC PTA 5289 was isolated from the oral cavity of a woman with good dental status despite poor oral hygiene.

As conditions of use, the applicant indicates that the food/constituent should be consumed twice daily as orodispersible lozenges containing live *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 in equal amounts (minimum of 1×10^8 CFU of each bacterial strain/lozenge). Different brand names are used for the same product depending on the country (e.g. BioGaia Prodentis, Gum Periobalance, Reladent or ReuterinOS). An overview of the manufacturing process and information regarding stability of batches was provided.

The Panel considers that the food/constituent, orodispersible lozenges containing a combination of live *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 in a 1:1 ratio, which is the subject of the health claim, is sufficiently characterised.

3.2. Relevance of the claimed effect to human health

The claimed effect proposed by the applicant is 'support normal gum function'. The proposed target population is 'the general population'.

Changes in gum structure leading to maintenance (i.e. reduced loss) of normal gum function(s) can be considered beneficial physiological effects. Evidence on whether (and the extent to which) specific changes in gum structure may lead to changes in gum function should be provided and will be considered on a case-by-case basis (EFSA NDA Panel, 2012).

Periodontal diseases comprise a wide range of inflammatory conditions that affect the supporting structures of the teeth (the gums, alveolar bone and periodontal ligament).

Gingivitis is a non-destructive, reversible condition consisting of the inflammation of the interdental and marginal gingival (gum) tissue without loss of the underlying supportive connective tissue. Gingivitis is most commonly caused by the attachment and growth of microbial species on teeth surfaces at or near gingival margins, forming dental plaque. Fungal and viral infections, as well as genetic factors, can play a role in the development of non-plaque-induced gingivitis. The onset and progression of plaque-induced gingivitis can be prevented by the accurate daily removal of dental plaque. In this context, dental plaque is a factor contributing to the development of gingivitis.

If untreated, gingivitis may become chronic and progress to periodontitis, which results in loss of connective tissue and bone support. Periodontitis is a non-reversible disease and a major cause of tooth loss in adults. The progression of gingivitis to periodontitis is difficult to predict as it depends on individual predisposition, local, systemic and exogenous factors.

The Panel considers that results from studies performed in subjects with gingivitis can be extrapolated to the target population for the claim (i.e. the general population). The Panel also considers that, since periodontitis is a chronic and non-reversible disease with permanent damage to periodontal tissues, the results from studies conducted in subjects with periodontitis cannot be extrapolated to the target population for the claim. Such studies, however, could be used as supportive evidence, if the outcome variables investigated are clinically relevant also for subjects with gingivitis in the absence of periodontitis.

Several outcome variables have been proposed by the applicant as appropriate to assess changes in gum function *in vivo* in humans (Pihlstrom et al., 2005; Lang and Bartold, 2018; Kumar, 2019). These are described below.

Bleeding on probing is an invasive, objective test, in which a periodontal probe is inserted into the gingival sulcus, (the recession of individual teeth) with a prespecified working pressure guided around the tooth. If a measurement position exhibits clinically visible bleeding within a specified time, normally in 10–30 seconds of probing, the BoP test is positive on that position. The number of positive sites is recorded, and the results are expressed as a percentage of the total number of sites examined (Bleeding Index). Gingival health can be defined as $< 10\%$ of bleeding sites with probing depths ≤ 3 mm. The pressure used for probing must be standardised because the percentage of sites with BoP increases linearly with an increase in probing force. The total number of teeth, their location, and number of sites per tooth probed should also be reported.

The Panel considers that BoP is an appropriate outcome measure to assess changes in gum structure leading to changes in gum function *in vivo* in humans in the context of the substantiation of health claims related to the maintenance of gum function. This outcome measure is clinically relevant for subjects with gingivitis and subjects with periodontitis.

Gingival index is a tool used for the evaluation of the inflammatory conditions of gingival connective tissues. The classical GI was originally introduced by Löe and Silness (Löe and Silness, 1963). Scores from 0 to 3 are assigned to each gingival site assessed. '0' means a normal gingiva, which is matt after drying, firm on palpation with a blunt instrument and presenting colours ranging from pale pink to pink; '1' refers to a mildly inflamed gingiva, which presents slight changes in colour or oedema but no BoP; '2' means moderate inflammation, displaying a red, reddish-blue or glazy gingiva and bleeding provoked on probing; '3' is the score for severe inflammation, the considered gingiva is markedly red or reddish-blue and enlarged, with tendency to spontaneous bleeding and ulceration. The GI score**,** a numeric continuous value ranging from 0.0 to 3.0, is obtained by averaging the scores assigned to all gingival sites in all teeth assessed. Therefore, the GI score includes both subjective, non-invasive (colour and contour of gums) and objective, invasive components (bleeding). Subjects with mild inflammation usually score from 0.1 to 1.0, those with moderate inflammation from 1.1 to 2.0, and a score between 2.1 to 3.0 means severe gingivitis. The modified gingival index (MGI), introduced by Lobene et al. (1986) is based on subjective, non-invasive components only (no probing). The MGI is less reliable and reproducible than BoP and GI because it does not ensure comparability between analyses due to the high variability of the measurements provided by different examiners (Martini et al., 2018).

The Panel considers that the classical GI is an appropriate outcome measure for the substantiation of health claims related to the maintenance of gum function, whereas the MGI could only be used as a supportive evidence. The information provided on intra- and inter-examiner calibration and blinding of the examiner(s) to the intervention is an important aspect to consider. GI and MGI are clinically relevant for subjects with gingivitis and subjects with periodontitis.

The plaque indices (PIs) are methods used to evaluate the plaque coverage without image capture. These methods are based on subjective visual evaluation and generally record either the absence or presence of dental plaque, or the extent and thickness near the gingival margin and the coronal extension of the plaque. Whereas some PIs may be appropriate methods to assess dental plaque in human intervention studies, both in subjects with gingivitis and in subjects with periodontitis, PIs are not direct measures of gingival structure or function.

The Panel considers that PIs are not appropriate outcome measures for the substantiation of health claims related to the maintenance of normal gum function. However, the Panel considers that plaque scores using well-established PI methods may be used in support of the mechanism by which the food/constituent could exert an effect on gingival structure and function, both in subjects with gingivitis and in subjects with periodontitis. The information provided on intra- and inter-examiner calibration and blinding of the examiner(s) to the intervention is an important aspect to consider.

Periodontal pocket depth (PD) and clinical attachment level (CAL) are clinical markers of loss of supporting gingival tissue and depth of the gingival sulcus. These outcome variables are mainly used for the diagnosis of periodontitis (PD $\geq$ 4 mm and CAL $\geq$ 4 mm), to assess the severity of periodontitis, to identify tooth that require treatment, and/or to assess the outcome of a treatment.

The Panel considers that periodontal PD and CLA are not appropriate outcome measures for the substantiation of health claims related to the maintenance of gum function because these outcome measures are only clinically relevant for subjects with periodontitis. However, these measures can be used as supportive evidence if assessed in subjects with gingivitis (and not periodontitis) together with BoP and/or GI.

The Panel considers that maintenance of normal gum function is a beneficial physiological effect.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search in PubMed on 30 November 2018 and an update on 2 April 2019 using the following key words: 'Lactobacill* reuteri AND (gingiv* OR bleeding on probing OR bop OR gums'. No restrictions were placed on publication date and language. A manual review was also performed to identify any additional relevant studies.

Eight human intervention studies (seven published and one unpublished) were identified as pertinent to the health claim. In all studies, lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 in a 1:1 ratio (minimum of 1×10^8 CFU of each bacterial strain/lozenge), the food/constituent which is the subject of the claim, were used. In five studies, one lozenge was consumed

by participants twice daily (as proposed in the conditions of use by the applicant), in two studies one lozenge per day was taken by participants (half of the recommended dose), and in one study the effect of both one and two lozenges per day was investigated.

Studies investigating BoP and the classical GI in subjects without periodontitis

Sabatini et al. (2017) in a randomised, parallel, two-arm study evaluated the effect of consuming lozenges containing *L. reuteri* twice daily on gingival function in subjects with type 2 diabetes mellitus (T2DM). Included were adults with T2DM and generalised gingivitis (BoP in at least 30% of sites) but no periodontitis (absence of PD > 4 mm; absence of CAL > 4 mm). Subjects with systemic diseases other than diabetes, uncontrolled diabetes, presence of periodontitis and smokers were excluded. The Panel notes that patients with T2DM are at higher risk of periodontal disease. However, they are considered as an appropriate study population for the claimed effect.

No professional teeth cleaning was performed. Participants were provided with toothbrushes and interproximal brushes and were instructed on how to brush their teeth.

Participants were randomised using a randomisation software. No information on allocation concealment was provided and no further clarification on this point was provided by the applicant upon EFSA's request. Participants in the intervention group were asked to consume a lozenge containing *L. reuteri* twice daily after toothbrushing, whereas participants in the control group received no intervention. All outcomes were measured at baseline and after 30 days. The Panel notes that the study was not blinded for participants and possibly not blinded for outcome assessors, as they had to be in close contact with participants for the evaluation of gingival outcomes.

The Panel notes that dietary intakes were not assessed in the study, that no information was provided about training and/or calibration of the examiner(s) performing the clinical tests, and that the methods used to assess compliance and adherence to the protocol were not reported.

Authors claim that sample size calculations were not performed in the absence of data available from other studies. A total of 40 participants per group was considered appropriate. BoP and PI were claimed to be the primary outcomes of the study. However, in the absence of power calculations, it is unclear whether these were selected *a priori* as primary outcomes. It was reported that BoP and PI were evaluated at six gingival sites per tooth. The Panel notes, however, that the number of teeth examined was not mentioned and no further information could be provided by the applicant following a request by EFSA.

It is reported that both differences within groups from baseline to day 30 and differences between groups were evaluated with the t-test for paired data. The Panel notes that the t-test for paired data is inappropriate to assess between-group differences in a parallel study (independent data), that the statistical analysis did not take into account baseline values and that the analysis was not corrected for multiple comparisons. The Panel also notes that the reported significance levels for between-group comparisons differed between text and tables (i.e. $p < 0.005$ and $p < 0.05$ respectively), and that it was not reported whether the measures of spread provided in the paper were standard deviations (SDs) or standard errors (SEs).

The Panel considers that, owing to important methodological limitations (i.e. study not blinded to participants and possibly not blinded to outcome assessors, no information on the training and/or calibration of the examiners performing the outcome assessment, number of teeth investigated not reported, lack of information on the assessment of compliance and adherence to the protocol, inappropriate statistical analysis), and the inconsistent reporting of the results, no conclusions can be drawn from this study for the scientific substantiation of the claim.

In a randomised, placebo-controlled, double-blind, two-arm, parallel study, Schlagenhauf et al. (2019) assessed the effect of consuming one lozenge containing *L. reuteri* twice daily on gingival outcomes in navy sailors, members of a ship, during a 6-week military exercise mission at sea.

Inclusion criteria were age 18–65 years, a minimum of 12 remaining natural teeth, the presence of the Ramfjord teeth (six teeth; 16, 21, 24, 36, 41 and 44; selected to represent the entire dentition) or their replacements, and at least one Ramfjord tooth showing BoP of the gingival sulcus. Exclusion criteria were regular use of anti-inflammatory medications possibly interfering with gingival inflammation, regular use of antimicrobial mouth rinses, gels or comparable medications, use of antibiotics ≤ 4 weeks prior to study participation, presence of a systemic disease interfering with gingival health (e.g. diabetes), and history of alcohol/drug abuse. The participants were instructed to maintain their dental care habits during the study. No other preventive dental actions were performed.

The primary outcome of the study was BoP, which was evaluated by probing six sites for each Ramfjord tooth. Secondary outcomes were MGI, PI, PD and CAL. All the outcomes were measured at baseline, at day 14 (intermediate re-evaluation) and at day 42 (final examination) of the study. All

examinations were performed by one trained examiner. Individual intake of sweet snacks was documented by a self-reported sugar consumption questionnaire. Main meals consumed by subjects were those served by the galley of the ship.

Sample size calculation was performed assuming a mean (±SD) number of 18 (±9) BoP-positive sites in the placebo group at the end of the study. In order to detect a reduction of BoP-positive sites in the test group of ≥ 50% with a power of 0.9 and α of 0.05, a sample size of 27 participants per arm was calculated. Taking into consideration potential drop-outs and protocol violations, 36 participants were recruited per arm for the trial.

A total of 72 of sailors displaying the highest score of gingival inflammation (out of 181 sailors who met the inclusion criteria) were randomised to consume either a lozenge with *L. reuteri* (n = 36) or a placebo lozenge (n = 36) twice daily during 42 days by slowly melting them on the tongue. Block randomisation (block size of four, stratified by smoking habits) followed the sequence of a computer-generated randomisation list. Compliance was assessed by counting the remaining lozenges in the assigned bottles brought back to the intermediate re-evaluation and final visits at days 14 and 42.

There were six drop-outs in the test group and four in the placebo group. Reasons were reported and unrelated to the intervention. A total of 68 participants (33 in the test group, 35 in the placebo group) were available for analysis at day 14, and 62 participants (30 in the test group, 32 in the placebo group) were evaluated at day 42. Five participants finished the trial with protocol violations (i.e. stopped consuming the lozenges during the trial or took antibiotics between visits at days 14 and 42). The statistical analyses were conducted for participants who were re-evaluated at least once at day 14 (33 in the test group and 35 in the placebo group).

Analysis of covariance (ANCOVA) with baseline values as covariate was used to assess differences between the groups at each time point (days 14 and 42).

The percentage of BoP-positive sites (Bleeding Index) was not significantly different between groups at baseline. On days 14 and 42, a significant reduction in the bleeding index was observed in the intervention group compared to placebo (mean difference in absolute changes from baseline, 95% CI: -22, -27 to -17% and -34, -40 to -28%, respectively). Similar results were observed when the analysis at day 42 was restricted to participants with a complete data set (mean difference, 95% CI: -34, -27 to -17%).

Significant reductions in GI, PI, PD and CAL were also observed in the intervention group compared to placebo ($p < 0.001$ in ANCOVA for all).

The Panel notes that the statistical analysis did not consider the repeated measures design of the study. However, the Panel also notes that accounting for this in the analysis would not have significantly changed the results considering the size of the effect observed.

The Panel considers that this study shows an effect of consuming one lozenge containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 in a 1:1 ratio (minimum of 1×10^8 CFU of each bacterial strain/lozenge) twice daily on BoP in subjects with gingivitis but no periodontitis. The results with respect to the other gingival outcomes investigated (i.e. MGI, PI, PD and CAL), which can be considered as supportive evidence, were consistent with the results on BoP.

Hasturk et al. (2015; unpublished study report) investigated the effect of consuming lozenges containing *L. reuteri* on gingival outcomes in a randomised, parallel, three-arm, placebo-controlled trial. Participants aged 18–65 years with mild to moderate chronic gingivitis were included. Exclusion criteria were periodontitis, medical conditions or on medications known to affect oral tissues, active infectious and/or inflammatory diseases or autoimmune diseases, use of antibiotics within the three months prior to enrolment, smoking, pregnancy or lactation.

After screening, eligible participants entered a washout period of 7–10 days to standardise their daily oral hygiene practices with a standard toothpaste and toothbrush. Then, participants were randomly allocated to one of the following study groups: one lozenge containing *L. reuteri* twice daily, one lozenge containing *L. reuteri* once daily, or one lozenge with no *L. reuteri* (placebo) daily. The Panel notes that, since the number of lozenges was different between the first intervention group and the placebo group, the study was not fully blinded. Insufficient information was provided to judge whether allocation of subjects to groups was appropriately concealed. The Panel also notes that dietary intakes were not assessed in the study.

The intervention lasted 12 weeks with a follow-up of another 12 weeks. The gingival outcomes were assessed at baseline and five times up to 24 weeks. Subjects were not allowed to use interdental cleaning devices, mouth rinses, whitening products, or chewing gums and were asked to abstain from all oral hygiene practices for the previous 12–18 hours before each visit. Compliance was evaluated at each visit and the participants with poor compliance or unwilling to adhere to the study protocol were withdrawn from the study.

A sample size of 18 subjects per group was calculated, based on reduction in MGI, with 80% power and $\alpha = 0.05$. Other outcome variables assessed were BoP, PD and PI. The number of sites and teeth examined was not reported. The two dental hygienists who performed all the dental tests were trained and calibrated for MGI assessments. Inter- and intra-examiner repeatability was checked before the initiation of the study. Although the authors claim that BoP and MGI were the primary outcomes of the study, the Panel notes that only MGI was used for power calculations and inter-examiner calibration.

Absolute values for gingival outcomes were calculated at each time point by averaging the scores obtained for each subject on all sites tested. Changes from baseline at each time point were obtained by calculating changes in the score within each specific site for each subject and then averaging them. Differences between groups in absolute values and in changes from baseline at each time point were assessed using three sets of independent t-tests (no correction for multiple comparisons) and analysis of variance (ANOVA) with a Bonferroni post-hoc test to correct for multiple comparisons.

A total of 70 subjects were randomised (n = 23, 24, and 23 to consume lozenges with *L. reuteri* twice daily, once daily, or placebo lozenges once daily, respectively; age 40 ± 13 years, 38 females) and 59 subjects finished the study. The reasons for drop-outs were given. Data from the 61 (n = 21 + 22 + 18, respectively) subjects who completed at least one post-baseline visit were used for the statistical analysis.

At baseline, there were no significant differences among groups in any of the variables measured (BoP, MGI, PD and PI). No significant differences between the *L. reuteri* groups (consuming lozenges twice and once daily, respectively) and placebo in relation to changes of BoP from baseline to 12 weeks (means ± SDs: −6.6 ± 14.9, 0.8 ± 13.5, 2.9 ± 14.5 respectively), were observed.

The Panel considers that this study with some limitations (not fully blinded, number of sites and teeth examined not reported) did not show an effect of consuming lozenges with *L. reuteri* once or twice daily for 12 weeks on BoP in subjects with gingivitis but no periodontitis. Results on MGI, PI and PD were consistent with the results on BoP. The Panel notes that the different results obtained in the study by Schlagenhauf et al. (2019) and in this study cannot be explained by differences in study design or sample size.

In a randomised, parallel, two-arm, placebo-controlled, double-blind study, Bravo et al. (2018) evaluated the effect of lozenges with *L. reuteri* on gingival outcomes in subjects with gingivitis aged 15–28 years. Gingivitis was defined as a GI ≥ 1.3 in more than 10% of the sites examined. Subjects with cavity lesions and/or defective restorations, on dental or periodontal treatment, on treatment with occlusal relaxation planes, with any disease or systemic condition affecting gingival tissues, on treatment with antibiotics and/or anti-inflammatories or any medicine that hinders gingival response in the 3 months prior to the intervention, were excluded.

The primary outcome was the classical GI. A sample size of 15 subjects per group was calculated considering a difference of 0.5 in GI changes between groups, a standard deviation of 0.23, an $\alpha = 0.05$ and a power of 80%. BoP and PI were secondary outcomes.

All the outcomes were assessed by a single calibrated examiner at the beginning and at the end (90 days) of the intervention. Intra-examiner calibration was performed showing good correlation between the results (0.86 for all outcomes measured). The number of sites and teeth examined were not reported.

Using computer-generated random number tables, 30 subjects were randomly assigned to consume one lozenge with *L. reuteri* or one placebo lozenge (identical without the bacteria) for 3 months (15 subjects per group). The randomisation was stratified according to sex, age (younger or older than 18 years), and smoking status. Allocation was concealed using opaque envelops. At baseline, all subjects underwent supragingival tartar removal and received oral hygiene instructions. All participants completed the study. Compliance was checked during weekly telephone calls and at the final visit by checking the number of unused lozenges. All participants were compliant. The Panel notes that the dietary habits were not assessed in the study.

Between-group comparisons were analysed with the Mann–Whitney U-test, Student's t-test and Fisher exact test. Upon a request from EFSA to clarify which test was used for which outcome variable, the applicant replied that ANOVA was used for BoP and GI. The Panel notes that an ANOVA analysis on two groups is equivalent to an independent t-test.

No statistically significant differences in BoP, GI or PI (both in absolute end values or as changes from baseline) were found between the test and control groups at the end of the intervention.

The Panel considers that this study with limitations (number of sites and teeth examined not reported, baseline values not included in the statistical analysis) did not show an effect of lozenges

with *L. reuteri* consumed once daily for 3 months on the classical GI or BoP in subjects with gingivitis but no periodontitis. The results on PI were consistent with the results on BoP and classical GI.

The Panel notes that three studies conducted in subjects with gingivitis but no periodontitis which assessed BoP and/the classical GI allowed conclusions to be drawn for the scientific substantiation of the claim, of which one study investigated the effect of lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 in a ratio 1:1 (minimum of 1×10^8 CFU of each bacterial strain/lozenge) when consumed twice daily under the proposed conditions of use (Schlagenhauf et al., 2019), one investigated the effect of lozenges given once daily only (Bravo et al., 2018), and one assessed both dosages (Hasturk, 2015). Out of the two studies that investigated the effect of the lozenges with *L. reuteri* consumed twice daily, one study (Schlagenhauf et al., 2019) showed an effect and one did not show an effect (Hasturk, 2015) on BoP. No effect on BoP or the classical GI was observed in the two studies which provided the lozenges once daily (Hasturk, 2015; Bravo et al., 2018). The results of other gingival outcomes in these three studies were consistent with the results reported for BoP and/or the classical GI within each study.

The Panel also notes that, although the effect of consuming lozenges containing *L. reuteri* twice daily on all the gingival outcomes measured was large as compared to placebo in the study by Schlagenhauf et al. (2019), no differences between lozenges with *L. reuteri* and placebo were observed in another study (Hasturk, 2015), and differences between the results obtained in these two studies could not be explained by differences in study design or sample size. No other studies were provided which had investigated the effect of lozenges containing *L. reuteri* on BoP and/or the classical GI in the target population (individuals without periodontitis) under the proposed conditions of use.

Other human intervention studies

Four human intervention studies conducted in adults (Tekce et al., 2015; Vivekananda et al., 2010; Iniesta et al., 2012; Schlagenhauf et al., 2016) were considered by the Panel as supportive evidence in relation to the claim, either because the studies investigated MGI (and not BoP or classical GI) in subjects with gingivitis but no periodontitis (Iniesta et al., 2012; Schlagenhauf et al., 2016) or were conducted in patients with periodontitis (Vivekananda et al., 2010; Tekce et al., 2015).

All four studies were double-blind, randomised controlled interventions in which outcomes were assessed by calibrated investigators. Lozenges were administered twice daily in three studies (Vivekananda et al., 2010; Tekce et al., 2015; Schlagenhauf et al., 2016) and once daily in one study (Iniesta et al., 2012). The duration of the intervention lasted between 3 and 7 weeks. Sample sizes ranged between 15 and 31 subjects per group. SRP prior to the intervention was performed in two studies (Tekce et al., 2015; split mouth design in Vivekananda et al., 2010). Subjects were either advised to continue their usual oral hygiene habits (Iniesta et al., 2012; Schlagenhauf et al., 2016) or received specific instructions on how to brush their teeth (Vivekananda et al., 2010; Tekce et al., 2015). The three studies providing lozenges twice daily showed an effect on either MGI in subjects with no periodontitis (Schlagenhauf et al., 2016) or BoP and the classical GI in subjects with periodontitis (Vivekananda et al., 2010; Tekce et al., 2015). In the study in which one lozenge per day was administered (Iniesta et al., 2012), no statistically significant differences were observed between groups with respect to MGI in subjects without periodontitis. The Panel notes, however, that these studies had methodological limitations regarding the statistical analyses performed (Vivekananda et al., 2010; Tekce et al., 2015; Iniesta et al., 2012), a high drop-out rate (Schlagenhauf et al., 2016), insufficient reporting on the number and location of the teeth tested, and on the number of sites examined (Vivekananda et al., 2010; Iniesta et al., 2012; Tekce et al., 2015).

The Panel considers that the results of the three studies which provided lozenges with *L. reuteri* twice daily (Vivekananda et al., 2010; Tekce et al., 2015; Schlagenhauf et al., 2016) support an effect of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 on gingival outcomes under the proposed conditions of use. The Panel notes that one study providing one lozenge per day (Iniesta et al., 2012) did not show an effect on MGI.

Mechanisms of action proposed

Competitive exclusion and inhibition of other bacteria

L. reuteri ATCC PTA 5289 has been shown to have an inhibitory effect on *Fusobacterium nucleatum* (IDH 4186), *Porphyromonas gingivalis* (ATCC 33277), *Prevotella intermedia* (ATCC 25611) and *Actinobacillus actinomycetemcomitans* (SA 1398) *in vitro* (Hedberg et al., 2006). *L. reuteri* DSM 17938 is reported to produce reuterin, an antimicrobial compound inducing oxidative stress in cells and

inhibiting *in vitro* the growth of both Gram-positive and Gram-negative bacteria such as *Clostridium difficile* (Spinler et al., 2017). In addition, the applicant provided data on the survival of the bacterial combination that is the subject of the claim in the saliva and subgingival environment after intake (Çaglar et al., 2009; Çaglar et al., 2015; Romani Vestman et al., 2015).

The ability of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 to inhibit the growth and exclude bacteria potentially involved in the development of human gingivitis and periodontitis *in vivo* as a possible mechanism of action has been investigated in several human studies.

Two randomised parallel, controlled and double-blinded clinical trials conducted in patients with chronic periodontitis and gingivitis considered above as supporting evidence (Vivekananda et al., 2010; Tekce et al., 2015) investigated the effect of administering one lozenge containing *L. reuteri* twice daily in combination with SRP on the microbiota of periodontal pockets. Tekce et al. (2015) report a decrease in total viable cell counts and the proportions of obligate anaerobes in the intervention group compared to placebo. Specific bacterial strains were not identified in this study. Vivekananda et al. (2010) report a decrease in bacterial species presumably related to gingivitis and periodontitis (e.g. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*) with *L. reuteri* lozenges vs placebo, both given alone or in combination with SRP. Gingival outcomes were also significantly reduced in the intervention group as compared to placebo in these two studies. Conversely, in the study by Iniesta et al. (2012) conducted in subjects with gingivitis but no periodontitis also considered as supporting evidence above, administration of one lozenge containing *L. reuteri* once daily did not affect total anaerobic counts at 4 or 8 weeks of intervention in subgingival samples. Counts of *P. gingivalis* and *A. actinomycetemcomitans* significantly differed between intervention and control groups at 4 weeks but not at 8 weeks. No significant differences between groups were observed in *Prevotella intermedia*, *F. nucleatum* and other five anaerobic bacteria strains at any time point. The Panel notes that the changes observed in the oral microbiota were inconsistent in this study and not accompanied by significant changes in the gingival outcome assessed (MGI).

In a randomised controlled trial, the effects of consuming lozenges containing *L. reuteri* twice daily for 12 weeks on saliva and dental biofilm microbiota were compared to placebo (n = 22 per group) (Romani Vestman et al., 2015). The analysis of saliva samples showed that lactobacilli counts assessed in agar plates and the prevalence of the *L. reuteri* strains DSM 17938 and ATCC PTA 5289 assessed by PCR were higher in the test group than in the placebo group. Also, a subset of tooth biofilm samples was analysed (only in 16 subjects randomly selected among the test (n = 8) and control (n = 8) groups) through 454-pyrosequencing of the 16SrDNA hypervariable regions. This analysis showed that, whereas the dental biofilm samples from the placebo group were stable over the 12-week intervention period, the composition of the microbiota of test group had changed after the intervention compared to baseline according to principal coordinate analysis (PCoA) of beta diversity. The intervention group showed a reduced proportion of *Streptococcus mutans* among the total streptococci and lower frequencies of the species *Neisseria mucosa*, *Fusobacterium periodicum*, *Fusobacterium nucleatum vincentii*, *Streptococcus anginosus* and *Prevotella maculosa* together with the presence of *L. reuteri* and a greater detection frequency of the species *Campylobacter concisus*, *G. adiacens*, *Bergeyella* sp. HOT322, *N. subflava*, R1 [G-1] sp. HOT874 and the *S. oralis*/*S. mitis*/*S. mitis* bv2/*S. infantis* group. Therefore, the intervention reduced the presence of bacterial species associated with caries (e.g. *S. mutans* seemed to be replaced by other *Streptococcus* species) and biofilm formation (*Fusobacterium* spp.) in the oral cavity. The Panel notes that gingival outcomes were not assessed in this study.

The Panel notes that in two other human intervention studies (Hasturk, 2015 and Schlagenhauf et al., 2019) conducted with *L. reuteri* lozenges consumed twice daily in patients with gingivitis and no periodontitis which assessed gingival outcomes, saliva and plaque samples were collected for microbiological assessment. Upon EFSA's request, the applicant explained that the samples collected have not been analysed.

The Panel notes that a significant decrease in some microbes potentially involved in human gingivitis in patients with periodontitis were observed in some human studies following consumption of lozenges containing *L. reuteri* twice daily (Vivekananda et al., 2010; Tekce et al., 2015), and that such changes were associated with a significant improvement in the gingival outcomes assessed. The Panel also notes that no evidence has been provided to establish that the same microbial strains are involved in the pathogenesis of gingivitis in subjects without periodontitis, or that changes in subgingival microbiota following the consumption of lozenges containing *L. reuteri* have an effect on gingival outcomes in subjects with gingivitis and no periodontitis (Iniesta et al., 2012; Romani Vestman et al., 2015).

Anti-inflammatory properties

In vitro, the combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 reduced *E. coli*-induced secretion of nuclear factor kappa-light-chain-enhancer of activated B cell (NFκB) transcribed pro-inflammatory cytokines TNF-α, IL-6, IL-8 and INF-ɣ in human macrophage-like U-937 cells (Brito et al., 2012). *L. reuteri* ATCC PTA 5289 supernatant enhanced prostaglandin E2 excretion in human gingival fibroblasts (Castiblanco et al., 2017). Biofilms supernatants of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 inhibited production of TNF-α in human monocytoid (THP-1) cells stimulated using lipopolysaccharide (Jones and Versalovic, 2009).

The applicant states that *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 have been shown to reduce pro-inflammatory cytokines, TNF-α and IL-8 in gingival crevicular fluid of patients with gingivitis.

In a study in 34 patients (Szkaradkiewicz et al., 2014) with chronic periodontitis and gingivitis who received professional cleaning of teeth accompanied by SRP, together with instructions to maintain hygiene of the oral cavity, patients assigned to treatment with *L. reuteri* ATCC PTA 5289 (n = 24) or placebo (n = 14) were identified on the basis of their response to the professional cleaning procedure (i.e. those with a 'statistically significant' decrease in clinical indices of gingivitis were assigned to placebo). Results on gingival outcomes and levels of TNF-α, IL-1β and IL-17 in saliva for patients in the intervention group were given separately for those showing a 'significant decrease' in mean values of gingival outcomes (n = 18) and those not showing a significant decrease (n = 6). Only within-group comparisons at the beginning and end of the intervention period are reported. The Panel notes that this study was not randomised, that the criteria for patient assignment to study groups, and for subgroup analyses, are unclear and not preplanned, and that the results from the intervention group are not compared to those of the placebo group. The Panel considers that no conclusions can be drawn from this study on the effects of *L. reuteri* ATCC PTA 5289 on either gingival outcomes or levels of TNF-α, IL-1β and IL-17 in saliva in patients with chronic periodontitis and gingivitis.

In the study by Schlagenhauf et al. (2016) conducted in pregnant women and considered above as supportive evidence, serum TNF-α concentrations differed at baseline between the intervention (receiving lozenges with *L. reuteri* twice daily) and placebo groups and were not significantly different at the end of the 7-week intervention. The Panel notes that this study shows no effect of the intervention on serum TNF-α concentrations. The Panel also notes that the relevance of this outcome (systemic TNF-α rather than TNF-α measured in subgingival or saliva samples) to the claimed effect is unclear.

In the study by Tekce et al. (2015) conducted in patients with gingivitis and periodontitis described above, matrix-metalloproteinase-8 concentrations and tissue inhibitor metalloproteinase-1 (TIMP-1) concentrations in gingival crevicular fluid were also measured in a subsample of 30 patients (İnce et al., 2015). The authors report a decrease in gingival crevicular fluid volumes and matrix-metalloproteinase-8 concentrations as well as increased TIMP-1 concentrations in gingival crevicular fluid. Metalloproteinase-8 is a collagenolytic proteinase that causes tissue destruction of the gingiva during periodontitis and correlates with the severity of periodontal disease, whereas tissue inhibitor metalloproteinases regulate metalloproteinase activity. The Panel notes that the relevance of this finding for subjects with gingivitis and no periodontitis is unclear.

In a randomised, double-blind, placebo controlled trial **(**Twetman et al., 2009**)** with chewing gum containing *L. reuteri* ATCC 55730 and *L. reuteri* ATCC PTA 5289 at two daily doses (1 or 2 $\times$ 10^8 CFU) for two weeks in 42 subjects (13 to 15 per group, three arms) with gingivitis, levels of IL-1b, TNF-a, IL-6, IL-8 and IL-10 were measured at baseline, 2 and 4 weeks in gingival cervical fluid. The follow-up values were compared to baseline within each group using non-parametric Wilcoxon paired two-sided test; differences between the groups were assessed using the Wilcoxon unpaired test. No significant differences between the intervention groups and placebo are reported in the publication at 2 or 4 weeks on these outcomes.

The Panel notes that no evidence has been provided to conclude that the consumption of lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 reduces markers of inflammation, which may be relevant for subjects without periodontitis.

Effects on barrier function

Karimi et al. (2018) showed that in an infection model based on the porcine intestinal cell line IPEC-J2, the pretreatment of the intestinal epithelial cells with *L. reuteri* DSM 17938 reduced the detrimental effect of enterotoxigenic *E. coli* in a dose-dependent manner, as monitored by transepithelial electrical resistance and translocation of fluorescein isothiocyanate dextran over the epithelial layer. The

Panel notes that the results from *in vitro* experiments performed in porcine intestinal epithelial cells cannot be directly extrapolated to what may occur *in vivo* in the human oral/gingival mucosa.

Conclusions on the mechanism of action

The Panel considers that, whereas some evidence has been provided for mechanisms by which consumption of lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 twice daily could improve outcomes of gingivitis in patients with periodontitis (i.e. exclusion of pathogenic bacteria from gingival pockets and possibly a reduction in local tissue damage), particularly if associated with professional plaque removal (SRP) in gingival pockets, the relevance of such mechanisms for the target population of the claim (i.e. subjects without periodontitis) is unclear.

Weighing the evidence

In weighing the evidence, the Panel took into account that, out of the two studies that investigated the effect of lozenges containing *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 on appropriate gingival outcomes (BoP and/or the classical GI) in subjects with gingivitis but without periodontitis under the proposed conditions of use (twice daily), one showed a large effect on BoP and other gingival outcomes (Schlagenhauf et al., 2019) and one showed no effect (Hasturk, 2015, unpublished). The inconsistent results obtained in these two studies could not be explained by differences in study design or sample size. The Panel notes that no other studies have been provided that could confirm the effect of consuming lozenges containing *L. reuteri* on appropriate gingival outcomes in the target population (subjects without periodontitis) under the proposed conditions of use. Among the four studies which investigated MGI (and not BoP or classical GI) in subjects with gingivitis, or were conducted in patients with periodontitis, three support an effect of lozenges with *L. reuteri* consumed twice daily on gum function under the proposed conditions of use. The Panel also took into account that, whereas some evidence has been provided for mechanisms by which consumption of lozenges containing *L. reuteri* twice daily could improve outcomes of gingivitis in patients with chronic periodontitis (i.e. exclusion of pathogenic bacteria from gingival pockets and possibly a reduction in local tissue damage), particularly if associated with professional plaque removal (SRP) in gingival pockets, the relevance of such mechanisms for the target population of the claim (i.e. subjects without periodontitis) is unclear.

The Panel concludes that the evidence provided is insufficient to establish a cause and effect relationship between the consumption of orodispersible lozenges containing combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 and maintenance of normal gum function.

4. Conclusions

On the basis of the data presented, the Panel concludes that:

- The food/constituent, orodispersible lozenges containing a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'support normal gum function'. The target population proposed by the applicant is 'general healthy population'. Maintenance of normal gum function is a beneficial physiological effect.
- The evidence provided is insufficient to establish a cause and effect relationship between the consumption of orodispersible lozenges containing a combination of *L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289 and maintenance of normal gum function.

Documentation as provided to EFSA

Health claim application on orodispersible lozenges containing combination of *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 and maintenance of normal gum function pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0485_SE). Submitted by BioGaia AB, P.O.Box 3242, SE 103 64 Stockholm, Sweden.

Steps taken by EFSA

1) This application was received by EFSA on 17/06/2019.
2) The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

3) The scientific evaluation procedure started on 30/07/2019.
4) On 10/09/2019, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 27/09/2019 and was restarted on 10/10/2019, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
5) On 29/10/2019, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application. The scientific evaluation was suspended on 21/11/2019 and was restarted on 29/11/2019, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
6) During its meeting on 22/01/2020, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to orodispersible lozenges containing combination of *Lactobacillus reuteri* DSM 17938 and *Lactobacillus reuteri* ATCC PTA 5289 and maintenance of normal gum function.

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Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATCC	American Type Culture Collection
BoP	bleeding on probing
CAL	clinical attachment level
CFU	colony forming unit
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
FITS-dextran	Fluorescein isothiocyanate-dextran
GI	gingival index
GMO	genetically Modified Organism
IL	interleukin
INF	interferon
MGI	modified gingival index
NDA	Nutrition, Novel Foods and Food Allergens
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
PAL	probing attachment level
PCoA	principal coordinate analysis
PCR	Polymerase Chain Reaction
PD	pocket depth
PI	plaque index
SD	standard deviation
SE	standard error
SRP	scaling and root planing
T2DM	type 2 diabetes mellitus
TIMP-1	tissue inhibitor metalloproteinase-1
TNF-α	tumour necrosis factor α