Bifidobacterium animalis subsp. lactis Bi-07 contributes to increasing lactose digestion: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

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Bifidobacterium animalis subsp. *lactis* Bi-07 contributes to increasing lactose digestion: evaluation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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 and Alfonso Siani

Abstract

Following an application from DuPont Nutrition Biosciences ApS submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Ireland, 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 *Bifidobacterium animalis* subsp. *lactis* Bi-07 (Bi-07) and contribution to increasing lactose digestion. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The food proposed by the applicant as the subject of the health claim is Bi-07. The Panel considers that Bi-07 is sufficiently characterised. The claimed effect proposed by the applicant is 'improvement of lactose digestion'. The Panel considers that increasing lactose digestion is a beneficial physiological effect for individuals with lactose maldigestion provided that the symptoms of lactose maldigestion are improved. Two human intervention studies which investigated a single dose effect of Bi-07 on lactose digestion using the hydrogen breath test, as well as on gastrointestinal symptoms were submitted. These studies show that consumption of Bi-07 (10^{12} CFU) increases lactose digestion in individuals with lactose maldigestion and that Bi-07 exhibits lactase activity *in vitro*. However, these studies provide no evidence that increasing lactose digestion through the consumption of Bi-07 (10^{12} CFU) improves gastrointestinal symptoms of lactose maldigestion, which is considered a beneficial physiological effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of *Bifidobacterium animalis* subsp. *lactis* Bi-07 and a beneficial physiological effect (i.e. the improvement of symptoms of lactose maldigestion) in individuals with lactose maldigestion.

Keywords: *Bifidobacterium animalis* subsp. *lactis* Bi-07, lactase, lactose maldigestion, health claim

Requestor: Competent Authority of Sweden following an application by DuPont Nutrition Biosciences ApS

Question number: EFSA-Q-2020-00024

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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.

Competing interests: A waiver was granted to an expert of the working group, Jean-Louis Bresson. Pursuant to Article 21(6) of the aforementioned 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: *Bifidobacterium animalis* subsp. *lactis* Bi-07 and contributes to increasing lactose digestion.

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of *Bifidobacterium animalis* subsp. *lactis* Bi-07, a positive assessment of its safety, nor a decision on whether *Bifidobacterium animalis* subsp. *lactis* Bi-07 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 '*Bifidobacterium animalis* subsp. *lactis* Bi-07 (Bi-07)'.

Health relationship as claimed by the applicant

According to the applicant, the health effect is related to 'improving lactose digestion'.

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

The applicant claims that: '(i) Bi-07 is able to digest lactose in simulated gastrointestinal environments, similarly or better than purified laboratory grade β-galactosidase, and better than other tested bacterial products (including standard yoghurt cultures); (ii) Bi-07 ($> 10^{12}$ CFU) is able to digest lactose similar to a target quantity of 4500 Food Chemicals Codex (FCC) of commercial lactase product originating from *Aspergillus oryzae*; (iii) the freely available enzymatic activity of Bi-07 is much lower than commercial lactase, demonstrating that Bi-07's mechanism of effect is due to bacterial metabolism and not by freely available enzyme'.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: '*Bifidobacterium animalis* subsp. *lactis* Bi-07 contributes to the improvement of lactose digestion in individuals who have difficulty digesting lactose'.

Specific conditions of use as proposed by the applicant

According to the applicant, the target population for the intended health claim is 'persons with lactose maldigestion'. The quantity of Bi-07 of at least 10^{12} CFU per serving is recommended.

Data provided by the applicant

The health claim application on '*Bifidobacterium animalis* subsp. *lactis* Bi-07 and contributes to the improvement of lactose digestion' pursuant to Article 13.5 of Regulation (EC) No 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 (EFSA NDA Panel, 2016a).

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, 2016a).

The scientific requirements for health claims related to the immune system, the gastrointestinal tract and defence against pathogenic microorganisms are outlined in a specific EFSA guidance (EFSA NDA Panel, 2016b).

The data claimed as proprietary are: Booster Omega Subject Classification, Booster Omega Safety Tables, Booster Omega Certificates of Analysis for Investigational Product, Booster Omega Clinical Study Report appendices and study reports by Forssten (2019 unpublished), Forssten and Marttinen (2019 unpublished) and Rasinkangas (2019 unpublished).

The data claimed as confidential are: Composition of *Bifidobacterium animalis* subsp. *lactis* Bi-07, specifications and details of test methods for Bi-07, certificates of analysis for three batches of Bi-07, manufacturing process of Bi-07, stability data of Bi-07, certificate of analysis present in the clinical study reports of the study by Uebelhack (2019 unpublished) and Donazzolo (2019 unpublished), certificate of analysis of Bi-07 in the *in vitro* studies by Forssten (2019 unpublished), Forssten and Marttinen (2019 unpublished) and Rasinkangas (2019 unpublished). EFSA has issued its Decision on Confidentiality on 26/05/2020.

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, 2016a). 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 *Bifidobacterium animalis* subsp. *lactis* Bi-07 (Bi-07).

Bi-07 is an anaerobic, Gram-positive, non-spore forming pleiotropic lactic acid bacteria (Ventura et al., 2007; Turroni et al., 2009; Arumugam et al., 2011).

The entire genome for Bi-07 has been sequenced (Stahl and Barrangou, 2012). The genome sequence has been deposited publicly at the National Center for Biotechnology Information (NCBI) (NC_017867.1) and in GenBank, the National Institute of Health's (NIH's) genetic sequence database (CP003498).

The strain has been deposited in the American Type Culture Collections (ATCC) safe deposit (SD5220), and in the internal Danisco Global Culture Collection (DGCC) (DGCC 2907). The species *Bifidobacterium animalis* was added to the EFSA's Qualified Presumption of Safety (QPS) List (EFSA BIOHAZ Panel, 2019). Bi-07 was accepted as Generally Recognised as Safe (GRAS) by the United States Food and Drug Administration (U.S. FDA, 2013).

An overview of the manufacturing process and information regarding stability of freeze-dried batches was provided (claimed as confidential information).

As conditions of use, the applicant indicates that the food/constituent should be consumed with lactose containing meals in an amount of at least 10^{12} colony-forming units (CFU) per serving.

The Panel considers that the food/constituent, *Bifidobacterium animalis* subsp. *lactis* Bi-07, 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 'improvement of lactose digestion'. The proposed target population is 'persons with lactose maldigestion'.

Lactose maldigestion results from a reduced enzymatic capacity to digest lactose. Individuals with lactose maldigestion may display symptoms after lactose consumption such as nausea, diarrhoea and gastrointestinal (GI) discomfort (e.g. cramping, bloating and flatulence) (EFSA NDA Panel, 2016b).

The scientific substantiation of health claims on improved lactose digestion that were assessed previously was based on human intervention studies showing an effect of the food/constituent on symptoms of lactose maldigestion (subjective outcomes), as well as an increase in lactose digestion (objectively measured by the breath hydrogen concentration method) when consumed with lactose-containing foods by individuals with symptoms of lactose maldigestion, and also on the biological plausibility of the effect. The characterisation of the study populations (i.e. individuals with symptoms of lactose maldigestion, irrespective of the cause) in the studies submitted for the substantiation of these claims is particularly important. Individuals with symptoms of lactose maldigestion could be identified through the appearance of symptoms upon lactose consumption and which respond to lactose withdrawal (EFSA NDA Panel, 2016b). Genetic testing may also be used as a first-stage screening test for individuals with lactose maldigestion.

The Panel considers that increasing lactose digestion is a beneficial physiological effect for individuals with lactose maldigestion provided that the symptoms of lactose maldigestion are improved.

3.3. Scientific substantiation of the claimed effect

The applicant performed a literature search on 24 September 2019 in the following databases: Allied & Complementary Medicine™, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA, MEDLINE® and NTIS (National Technical Information Service). Keywords used for Bi-07 were '*Bifidobacterium animalis*' or '*Bifidobacterium lactis*' or '*Bifidobacterium infantis*' or '*B. infantis*' or 'BBI' or 'B. lactis' or 'B. animalis' or 'Bi-07', 'Bi07', 'Bi 07', 'BI-07', 'BI07', 'BI 07' and terms related to outcome were 'lactose' or 'lactase'.

No pertinent human intervention studies were retrieved by the search. The applicant submitted two unpublished human intervention studies as pertinent to the health claim (Donazzolo, 2019 unpublished; Uebelhack, 2019 unpublished).

They were both randomised, double-blind, cross-over, three-sequence, placebo-controlled, positive-controlled studies which investigated a single-dose effect of Bi-07 on lactose digestion using the hydrogen breath test (HBT), and on gastrointestinal (GI) symptoms. All participants included in the

studies had lactose maldigestion as confirmed by genetic testing. The two studies were carried out using a similar design.

Upon a request from EFSA, the applicant clarified that both studies were designed to study the effect of Bi-07 on lactose digestion assessed by the HBT. The studies were not powered to assess the effect of Bi-07 on GI symptoms. The applicant stated also that lactose maldigestion (i.e. the presence of lactose in the colonic lumen) does not necessarily lead to lactose intolerance ('symptomatic lactose maldigestion'). Factors which determine whether undigested lactose causes lactose intolerance include the amount of lactose ingested, small intestinal lactase activity, gastric emptying rate, transit time and GI microbiota composition (Vesa et al., 2000; de Vrese et al., 2001; Montalto et al., 2005).

Individuals (aged 25–60 years, both sexes) with self-declared, suspected or medically diagnosed lactose maldigestion were recruited into the studies by advertisements, by verbal communication of the research centres staff, and with the use of the research centres databases. Lactose maldigestion was confirmed at a screening visit by the ingestion of a lactose solution (25 g lactose in water or fat-free milk) by participants and a subsequent increase in breath hydrogen with $\geq$ 20 ppm. This threshold was in line with the conclusions of a North American consensus document (Rezaie et al., 2017). Maximum mean ($\pm$standard deviation (SD)) breath hydrogen concentrations of included participants at the screening visits were 101 $\pm$ 47.1 ppm in the study by Donazzolo (2019 unpublished) and 73.1 $\pm$ 29.6 ppm in the study by Uebelhack (2019 unpublished). Lactose maldigestion was verified, in addition, in all participants during the study by a gene test that screened for the most common single nucleotide polymorphisms associated with lactose maldigestion (Enattah et al., 2002; Tishkoff et al., 2007). Individuals with an increase of less than 20 ppm in breath hydrogen within 3 hours from baseline were excluded from the study. People with GI diseases, with history of abdominal surgeries, with recent antibiotic treatment, with history of alcohol abuse and regular smokers were excluded.

Randomisation was performed using an online tool incorporated into the electronic case report forms (eCRF). Power calculation was based on a 35% decrease in the incremental area under the curve (iAUC; ppm x h) of breath hydrogen concentrations for Bi-07 compared with placebo (the expected effect was based on the results of a previously performed study by Sanders et al. (1992), assuming an SD of 0.7 and a 10% attrition rate. In order to reach 90% power at a significance level of 5%, 34 participants were needed and recruited into both studies.

In each study, three acute lactose challenges (25 g) were performed. One sachet containing either placebo (maltodextrin), 1.8×10^{12} CFU Bi-07, or 4500 Food Chemicals Codex (FCC) units of lactase with maltodextrin as a carrier, was mixed using a blender by study personnel with 250 mL of water and 25 g lactose (Donazzolo, 2019 unpublished), or with 521 mL of fat-free milk containing 25 g lactose (Uebelhack, 2019 unpublished) immediately before administration. The mixture was consumed by participants within prespecified time periods (i.e. in Donazzolo (2019 unpublished) within 30 seconds from preparation and in Uebelhack (2019 unpublished) within 5 minutes). The challenges were carried out in random order using the Williams design for cross-over studies with 7-day wash-out periods in between.

Breath gas (hydrogen, methane and carbon dioxide) measurements were performed immediately after the ingestion of the study products (after 5 min from the start of the challenge) and every 30 minutes for 6 hours after the start of the challenge.

GI symptoms were assessed with the use of a non-validated questionnaire which was filled in 15 min before the start of the challenge, 5 min after the start of the challenge and every 60 minutes from the start of the challenge until the end of the monitoring period. Abdominal pain, flatulence, bloating and nausea were assessed by using a 4-point Likert scale (none, mild, moderate, severe), vomiting, bowel movements and diarrhoea were rated as absent or present. If bowel movements and/or diarrhoea were rated as present, the Bristol stool scale was used to assess stool consistency and a number of bowel movements were recorded. The Panel notes that the assessment method of GI symptoms was not validated. The Panel also notes that 4-point Likert scales are commonly used in the assessment of GI symptoms and that symptoms were evaluated individually without combining them into an overall GI symptom score that would have required validation.

The primary outcome measure of the study was the difference in breath hydrogen concentrations between (1) the Bi-07 and the placebo periods, (2) the lactase and the placebo periods and (3) a non-inferiority analysis between the Bi-07 and the lactase periods.

Secondary outcome measures included breath hydrogen peak values, cumulative breath hydrogen values and the severity of abdominal pain, flatulence, bloating, nausea, vomiting, bowel movements and diarrhoea.

The multiple testing strategy for the primary outcome followed a fixed-sequence testing strategy in the order indicated above.

In the statistical analysis, iAUC was analysed on the natural log-transformed scale. The linear mixed model that was used included baseline breath hydrogen concentrations as a covariate and sequence, period and treatment as fixed effects and participant within sequence as random effect. Results are presented as ratios of geometric least squares means (LSM) and the associated 95% CIs. A two-sided 95% CI for the difference (Bi-07 – lactase) was applied in the evaluation of the non-inferiority hypothesis. The non-inferiority margin was set at 1.25. Carry-over effects were evaluated separately by adding a first-order carry-over effect into the model described above.

The presence of bowel movements and diarrhoea was analysed using a logistic regression model with random intercepts. The period-wise maximum severity of abdominal pain, flatulence, bloating and nausea was analysed using mixed effects cumulative logit-models for ordinal responses and logistic regression for modelling the odds of higher ordered severities (i.e. at least moderate). Stool consistency was evaluated descriptively.

The primary analysis was conducted on the per protocol (PP) population. The intention-to-treat (ITT) population was used to support the primary evaluations.

In Donazzolo (2019), 34 participants were randomised (68% women, mean age 49.2 ± 11.3 years). All of them finished the study. Two participants vomited during the Bi-07 challenge and both were excluded from the PP population.

There was a statistically significant sequence effect in the study. According to the authors of the study report, this was mainly due to two sequences (Bi-07-lactase-placebo and placebo-lactase-Bi-07) which showed lower mean breath hydrogen concentrations than the other sequences.

In the PP population, there was a statistically significant decrease in the iAUC for breath hydrogen in the Bi-07 compared with the placebo period (mean values of iAUC ± SD: 134.7 ± 120.76 vs. 235.7 ± 142.80 ppm/hour; geometric LSM ratio 0.227 (95%CI 0.095; 0.543); p = 0.0012). There was no statistically significant difference between lactase and placebo (162.6 ± 111.92 vs. 235.7 ± 142.80 ppm/hour; 0.493 (0.210; 1.156); p = 0.1022). The non-inferiority of Bi-07 vs. lactase was shown as the upper limit of the geometric LMS ratio 95% CI (1.096) and was below the non-inferiority margin of 1.25. The Panel notes that the results of the non-inferiority analysis cannot be interpreted in the absence of significant differences between lactase and placebo. The results of the ITT analysis were in line with those of the PP population.

There were no statistically significant differences between Bi-07 and placebo or Bi-07 and lactase in the odds of developing at least moderate abdominal pain, flatulence and bloating. The odds of higher severity classes of nausea were significantly increased in the Bi-07 period compared with placebo (OR 4.31 (95%CI 1.57; 11.85) or with lactase (6.98 (2.42; 20.1). The Panel notes the large uncertainty around the effect and that the results were not corrected for multiple testing of outcomes. The Panel also notes that the study was not designed to investigate GI symptoms.

The Panel considers that this study shows an effect of a single dose of Bi-07 (10^{12} CFU) suspended in water with 25 g lactose on lactose digestion assessed by HBT in individuals with lactose maldigestion. However, the increased lactose digestion was not associated with a reduction of GI symptoms of lactose maldigestion in the same individuals.

In Uebelhack (2019 unpublished), 34 participants were randomised (56% women, mean age 37.7 ± 11.1 years), and all of them completed the study. One individual was *ex post* excluded from PP analysis because of the regular use of tobacco (that was an exclusion criterion).

Results from this study also showed a significant sequence effect for one of the sequences (placebo-Bi-07-lactase), which was different from the sequences for which a significant sequence effect was found in the study by Donazzolo (2019 unpublished). According to the author of the study report, this was due to two outliers whose breath hydrogen iAUCs were zero after lactase administration. Removing these two participants in a sensitivity analysis led to the results of the analysis of the sequence effect to become non-statistically significant.

In addition, a carry-over effect was observed. The author of the study report explained that in six participants, Bi-07 was detected in faeces in the placebo period that was preceded by the Bi-07 administration. In this period, they also had breath hydrogen iAUCs that were exceptionally low. These low iAUCs could, according to the author, be explained by the carry-over effect that was observed.

In the PP population, iAUC for breath hydrogen was statistically significant lower in the Bi-07 period compared with placebo (mean values ± SD: 172.5 ± 86.33 vs. 219.4 ± 101.21 ppm/hour; geometric LSM ratio 0.462 (95% CI 0.249; 0.859); p = 0.0156). Significant differences (i.e. lower iAUC) were also found for lactase vs. placebo (mean values ± SD: 123.0 ± 121.20 vs. 219.4 ± 101.21; geometric LSM

ratio 0.190 (0.102; 0.365); $p < 0.0001$). The non-inferiority of Bi-07 vs. lactase was not shown as the upper limit of the geometric LSM ratio's 95% CI (4.55) was above the non-inferiority margin of 1.25. The results of the ITT analyses were in line with those of the PP population.

There were no statistically significant differences between Bi-07 and placebo or Bi-07 and lactase in the odds of higher severity classes (i.e. at least moderate) bloating and nausea. The odds of higher severity classes of abdominal pain and flatulence were significantly increased in the Bi-07 period compared with lactase (OR 3.13 (95%CI 1.10; 8.87) and 3.4 (1.29; 9.00), respectively, but not compared with placebo (1.02 (0.38; 2.74) and 0.86 (0.34; 2.19), respectively. The Panel notes the large uncertainty around the effect and that the results were not corrected for multiple testing of outcomes. The Panel also notes that the study was not designed to investigate GI symptoms.

The Panel considers that this study shows an effect of a single dose of Bi-07 (10^{12} CFU) suspended in fat-free milk containing 25 g lactose on lactose digestion assessed by HBT in individuals with lactose maldigestion. However, the increased lactose digestion was not associated with a reduction of GI symptoms of lactose maldigestion in the same individuals.

Proposed mechanism of action

The applicant claims that Bi-07 exhibits lactase activity. The lactase activity of Bi-07 was determined in three *in vitro* studies: by assessing lactase activity of Bi-07 itself (Rasinkangas, 2019 unpublished; Forssten, 2019 unpublished) and indirectly by assessing residual lactose content (Forssten and Marttinen, 2019; unpublished).

The study conducted by Rasinkangas (2019 unpublished) was carried out as part of a quality control and stability analysis of the products used in each of the clinical studies described above (Donazzolo, 2019 unpublished; Uebelhack, 2019 unpublished). In this study, the lactase activity of Bi-07 was 23.0 FCC/g and 23.6 FCC/g in the refrigerated reference samples of the batches used in Uebelhack (2019 unpublished) and Donazzolo (2019 unpublished), respectively. Analyses of refrigerated returned samples from the study sites yielded a lactase activity of 28.6 FCC/g and 32.2 FCC/g, respectively. In comparison, the activity of lactase administered in the studies ranged from 777 to 871 FCC/g. The low levels of lactase activity measured in the Bi-07 samples were explained by the author to be due to the location of the lactase enzyme within the viable cells of the bacteria. The increase in measured FCC/g for Bi-07 is thought to be due to a reduction in the viability over time, which may result in the release of the enzyme from the cells. The increase in the activity of the lactase samples was explained by measurement uncertainty.

Forssten (2019) assessed the lactase activity of different bacterial strains, among them Bi-07, either when lactose was added to sealed tubes containing the cultures of the strain (experiment 1) or in simulated human upper GI tract conditions (experiment 2). The lactase activity was measured by comparing to standards of a p-nitrophenyl-b-d-galactopyranoside standard curve. The activity of Bi-07 was similar to several yoghurt cultures (around 1.1 nmol/g/min) in the first experiment, including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, which have been evaluated with a favourable outcome for their effect on lactose digestion by the Panel (EFSA NDA Panel, 2010). In experiment 2, Bi-07 showed, using descriptive statistics only, a higher lactase activity than the above-mentioned yoghurt cultures both in the stomach and the duodenal simulations and higher activity compared with the purified lactase in the duodenal stage.

Forssten and Marttinen (2019) assessed the lactase activity of Bi-07 in fat-free milk, as measured by the residual lactose content. Fat-free milk (500 mL) at 37°C was exposed to a dose of 6 g of freeze-dried Bi-07 (1.8×10^{12} CFU), placebo (6 g maltodextrin) or 4,500 FCC of lactase (two different samples: food grade and laboratory grade). Samples were collected during 6 h of incubation with 30-min intervals during the first hour and thereafter with 1-h intervals. In order to stop the enzymatic activity, the samples were placed for 10 min in a water bath at 98°C. The lactose content of samples was determined using a UV-method. Bi-07 caused a decrease of the lactose amount that was similar to the two lactase samples, while for the untreated milk and the placebo samples, there was no change in the amount of lactose during the 6-hour measurements. The difference between Bi-07 and placebo was statistically significant in RM-ANOVA analysis.

The Panel considers that the evidence provided in the *in vitro* studies, either assessed directly by measuring the enzymatic activity, or indirectly by assessing residual lactose content, shows that Bi-07 exhibits lactase activity.

Weighing the evidence

In weighing the evidence, the Panel considered that the two human intervention studies provided show that consumption of Bi-07 (10^{12} CFU) increases lactose digestion in individuals with lactose maldigestion and that Bi-07 exhibits lactase activity *in vitro*. However, the Panel also considered that these studies provide no evidence that increasing lactose digestion through the consumption of Bi-07 (10^{12} CFU) improves GI symptoms of lactose maldigestion, which is considered a beneficial physiological effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of *Bifidobacterium animalis* subsp. *lactis* Bi-07 and a beneficial physiological effect (i.e. the improvement of symptoms of lactose maldigestion) in individuals with lactose maldigestion.

4. Conclusions

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

- The food/constituent, *Bifidobacterium animalis* subsp. *lactis* Bi-07, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is 'improvement of lactose digestion'. The target population proposed by the applicant is 'persons with lactose maldigestion'. Increasing lactose digestion is a beneficial physiological effect for individuals with lactose maldigestion provided that the symptoms of lactose maldigestion are improved.
- A cause and effect relationship has not been established between the consumption of *Bifidobacterium animalis* subsp. *lactis* Bi-07 and a beneficial physiological effect (i.e. the improvement of symptoms of lactose maldigestion) in individuals with lactose maldigestion.

Documentation as provided to EFSA

Health claim application on *Bifidobacterium animalis* subsp. *lactis* Bi-07 contributes to the improvement of lactose digestion in individuals with lactose maldigestion pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 0492_IE). Submitted by DuPont Nutrition Biosciences ApS Langebrogade 1 DK-1411, Copenhagen K, Denmark.

Steps taken by EFSA

1) This application was received by EFSA on 8/01/2020.
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 26/02/2020.
4) On 27/02/2020, 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 12/03/2020 and was restarted on 26/03/2020, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
5) During its meeting on 01/07/2020, the NDA Panel, having evaluated the data, adopted an opinion on the scientific substantiation of a health claim related to the consumption of *Bifidobacterium animalis* subsp. *lactis* Bi-07 and improved digestion of lactose in individuals with lactose maldigestion.

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Abbreviations

ATCC	American Type Culture Collection
CFU	Colony Forming Unit
CI	Confidence Interval

DGCC	Danisco Global Culture Collection
FCC	Food Chemicals Codex
GI	gastrointestinal
GRAS	Generally Recognised As Safe
HBT	hydrogen breath test
iAUC	incremental Area Under the Curve
ITT	Intention To Treat
LSM	least squares means
NCBI	National Center for Biotechnology Information
NDA	Panel Panel on Nutrition, Novel Foods and Food Allergens
NIH	National Institute of Health
NTIS	National Technical Information Service
PP	Per Protocol
RM	ANOVA Repeated Measures-Analysis of Variance
SD	Standard Deviation
UV	Ultraviolet