

Horizons in Nutritional Science

Health benefits and health claims of probiotics: bridging science and marketing

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Abstract

Health claims for probiotics are evaluated by the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority. Despite a substantial amount of basic and clinical research on the beneficial effects of probiotics, all of the evaluated claim applications thus far have received a negative opinion. With the restrictions on the use of clinical endpoints, validated biomarkers for gut health and immune health in relation to reduction in disease risk are needed. Clear-cut criteria for design as well as evaluation of future studies are needed. An open dialogue between basic and clinical scientists, regulatory authorities, food and nutrition industry, and consumers could bridge the gap between science and marketing of probiotics.

Key words: Probiotics; Health claims; Regulatory authorities

The European Regulation on Nutrition and Health Claims of 2006⁽¹⁾ provides a common regulation allowing health claims to be made on foods in a uniform manner throughout the member states in the European Union (EU). The main objectives of the EU regulation were to ensure a high level of consumer protection, to allow effective functioning of the internal market within the EU, to increase legal security for economic operators, fair competition within the food and nutrition industry, and stimulation and protection of innovations in the food sector. When fully implemented, only health claims obtained on the community list of permitted health claims will be allowed within the EU. The European Food Safety Authority (EFSA) has received 4637 health claim submissions, of which 8% deal with probiotics when the existing health claims were submitted by the member states in 2007. The current situation (November

2010) is that the Panel on Dietetic Products, Nutrition and Allergies (NDA) of EFSA has expressed a negative opinion on all of the claims on probiotics evaluated thus far. Thus, the objectives of the regulation are invalidated with respect to probiotics: rejection of all claims does not improve consumer protection, hinders cross-border movements of goods, has a negative impact on the willingness of companies to invest in research and development, and does not stimulate fair competition. Most importantly, the objective of protecting consumers is not met. The consumer does not receive adequate information on probiotics, in which a considerable amount of research and development has been invested.

Health claims for food (including probiotics) can be submitted under either article 13 or 14. Claims that relate to growth, development and function of the body (including

Abbreviations: EFSA, European Food Safety Authority; EU, European Union; IBS, irritable bowel syndrome; NDA, Panel on Dietetic Products, Nutrition and Allergies; NK, natural killer.

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immune function); psychological and behavioural functions; or weight management belong to article 13, and claims that relate to a reduction in disease risk or refer to children's development and health must be submitted under article 14. Health claims related to article 13.1 must be based on generally accepted scientific evidence. When based on newly developed scientific evidence, the claims belong to article 13.5, which may include protection of proprietary data.

Probiotics are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host'⁽²⁾. The health claim is already incorporated in the definition of probiotics: confer a health benefit. Thus, as long as the consumer would take in adequate amounts and the micro-organisms are alive, a generic health claim could be made, as long as a benefit has been demonstrated. The WHO definition of probiotics is the favoured one, because it is used by the US Food and Drug Administration, recommended by the International Scientific Association for Probiotics and Prebiotics and is used in the majority of the scientific publications. It is stated that the use of the term 'probiotic' in itself thus corresponds to a health claim but is not acceptable by EFSA, as the claimed effect is not measurable⁽³⁾.

Over the last two decades, there has been a growing interest on both basic and clinical science in probiotics. This has resulted in more than 6000 publications in the biomedical literature, with over 60% published since 2008 including articles in the highest-ranking medical and basic scientific journals. With regard to the clinical and nutritional application of probiotics, guidelines have been proposed to establish and substantiate their beneficial effects, including design of randomised clinical trials⁽⁴⁾. The PASSCLAIM project was carried out to define the normal function of the gut and immune system and describe available methods of measuring this function⁽⁵⁾. A great number of well-designed and well-conducted trials have been published, substantiating the beneficial effects of (specific strains of) probiotics on risk reduction and management of a variety of diseases and conditions. When performing a literature search on probiotics, PubMed suggests to use diarrhoea, irritable bowel, immune, prevention, treatment, allergy, clostridium, intestinal and ulcerative as additional search terms. For the combination of key words 'probiotics', 'clinical trial' and 'human', over 750 publications are listed, many of which are published in top ranking nutrition and clinical journals. The translation of this considerable amount of basic and clinical research into practical conclusions (and approved health claims) apparently goes wrong. Why? At present, there are gaps between biomedical science and regulatory requirements for getting a health claim approved. In this paper, we will illustrate some of the gaps that prevent approval of health claims.

Suppose a study (randomised, controlled and double-blind) has been performed in healthy adult volunteers (50–70 years old) who consume either the (imaginary) probiotic strain '*Lactobacillus prosana*' or placebo for 30 d. Comparison of blood natural killer (NK) cell activity measured before and after the intervention shows a significant increase in NK activity in the probiotic group and no change in the placebo group. An independent replication of this study has confirmed the results. What health effect can be claimed?

- (1) *L. prosana* enhances NK cell activity.
- (2) *L. prosana* improves natural immunity against viruses.
- (3) *L. prosana* protects against viral infections.

From the scientific perspective, the first answer is the correct one, but from a regulatory perspective, all answers to this hypothetical multiple choice question are wrong. EFSA has listed the regulatory issues that should be addressed when making a health claim. Minimally these are (1) characterisation of the probiotic product, (2) substantiation of the immune health benefit, i.e. demonstration that the biomarker measured contributes to an improvement of the overall functioning of the immune system and (3) extrapolation to the general healthy population. These regulatory issues, which are required for bridging probiotics science and probiotics marketing, have been pivotal in the evaluation of health claims for probiotics.

Characterisation of probiotic bacteria

It is a well-established fact that the biological effects of probiotics are strain specific. Stig Bengmark has made this very clear in his statement that the (genetic) difference between one probiotic bacterium and the other is larger than the difference between a man and a goldfish. The success (or failure) of one strain cannot be extrapolated to another strain (or strains). The strain-specific benefits of probiotics thus emphasises the need for proper strain identification⁽⁶⁾.

Unambiguous species identification can be performed by 16S rRNA gene sequence analysis and by DNA–DNA hybridisation techniques. For strain identification, different molecular microbiological typing techniques can be applied such as pulsed-field gel electrophoresis, amplified fragment length polymorphism or multilocus sequence typing. It would be helpful if the regulatory authorities, together with organisations such as the International Life Science Institute, the International Dairy Federation, the European Food and Feed Culture Association, and others in the scientific community could assign one, or several, of these techniques as necessary and sufficient for strain identification. Moreover, genomic sequence technology is developing rapidly and will become available soon and can facilitate strain identification.

For both scientific and regulatory purposes, bacteria should be named according to the international nomenclature rules, and fantasy names such as *L. prosana* are not allowed. Furthermore, strains should be deposited at internationally recognised culture collections with access numbers. In future scientific publications, these details on the characterisation and identification of the probiotic strains could already be included (or referred to as supplementary data).

The techniques used for strain identification generate a 'fingerprint' of a particular bacterial strain. These techniques do not provide information on the structure or sequence of functionality encoding genes. Thus, the genetic fingerprint only serves regulatory purposes and bears no relation to functionality. Strains that appear to differ in molecular typing may have identical functionality encoding genes⁽⁴⁾. On the other hand, the mere presence of a given gene is also insufficient to ascertain a specific health benefit, as there is a 'carrier' impact

of the strain, i.e. the same gene in different species may not lead to an identical effect. Unfortunately, with a few exceptions^(7,8), the genes that determine or underlie the health benefit delivered by specific probiotic strains have not been identified to date. There are no *in vitro* assays or even animal models, which allow to establish a probiotic effect, but they might be used to characterise the probiotic or to establish possible mechanisms of action. Demonstration of a beneficial effect of a given probiotic strain can only be demonstrated in functional *in vivo* studies. The implication is that every new strain or combination of strains should be subjected to costly and lengthy clinical studies to substantiate a health claim.

Health benefits in healthy people

The WHO has defined health as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity⁽⁹⁾. This definition was proposed in 1948 at the inauguration of the WHO and has not been revised in over 60 years.

In relation to health claims, the operational value of the WHO definition is limited because it leaves no room for improvement (a state of complete ... well-being), and thus it would be impossible for probiotics or any other nutrient to have a beneficial effect in healthy individuals. The present definition of health as a state lacks the temporal dimension and does not address its dynamics. Yet, the health state will have its oscillations, depending on changes in intrinsic factors (human biology and lifestyle) and extrinsic factors (the environment and healthcare services). Thus, in order to maintain health, constant adaptation and response to challenge are required, which depends on the resilience capacity (i.e. the capacity to return to a homeostatic state) of the individual subject. An example is antibiotic-associated diarrhoea, which will develop when the resilience capacity is insufficient to maintain the equilibrium of the gut microbiota (see also below). Outside the food and nutrition area, the term 'enhancement' is used for methods to improve the healthy body. Compounds that improve brain function, such as nootropics, referred to as smart drugs, are examples of enhancement. The whole field of preventive medicine, such as childhood vaccination programmes and addition of fluoride to drinking-water, to prevent caries and maintain oral health, deals with the maintenance of health. The objective of vaccination is to confer protective immunity (by stimulation of the immune system) against an otherwise severe infectious disease. Vaccines are administered to healthy children and protective effects are evident at the population level as a reduction in the incidence of disease, in most cases by >98%. For an individual child, it is more difficult to estimate whether the child is benefited from the herd immunity or whether the child's immune system could have been strong enough to combat the infection in question.

Function of the immune system in relation to human health

The function of the immune system is to protect against infectious diseases. In order to fulfil this function, the immune

system operates at two levels. The innate immune response is activated within minutes or hours of encountering the antigen, and the specific adaptive immunity takes days or weeks to develop and act. Phagocytes and NK cells produce cytokines and chemokines, which together with complement lead to the innate responses. Innate immunity orchestrates adaptive immunity that consists of B and T lymphocytes responsible for antibody production and generation of cytotoxic T lymphocytes. The capacity of the adaptive immune system to develop immunological memory is utilised in vaccination to prevent infectious diseases. Under normal physiological conditions, the immune system protects the individual against most infections. The function of the immune system can be temporarily impaired due to intrinsic or extrinsic factors, can be permanently weakened for example in the elderly and is not yet fully mature during infancy. Even short periods of reduced immune competence can compromise the ability to fight infections.

Probiotics have the capacity to stimulate various components of the immune system. In 2005, Albers *et al.*⁽¹⁰⁾ identified twenty-eight different groups of markers to measure immunomodulation in human nutrition intervention studies. Ig levels in serum and at mucosal surfaces, systemic cytokine concentrations, number and activity of phagocytic cells and NK cells are among the endpoints most often studied. However, none of these biomarkers are by themselves indicative for the overall immune status of an individual. Therefore, it is difficult to predict whether for instance a 25% increase in the concentration of secretory IgA in the mucosal surfaces of the upper respiratory tract really would confer better protection against influenza virus infection. This is the reason why claims such as 'strengthens the immune system' are considered too vague or even meaningless, unless they relate to a more specific health claim such as 'reduces the risk of common cold episodes during winter season'.

There is a clear gap between the scientific understanding of the immune markers and immune system and the regulatory requirement for measuring the effect on immune health. In order to be able to substantiate an immune health claim appropriately, a list of markers relevant for immunity published by EFSA would be of great importance. For the time being, double-blind placebo-controlled (DBPC) clinical trials on the reduction of disease risk that also incorporate immune markers would be required.

In order to demonstrate that probiotics can actually reduce the risk for gastrointestinal and respiratory infections, DBPC clinical trials are required. To that end, in most cases, the study population consists of subjects with an increased risk of infection, namely the (very) young and the (very) old. The frequency of respiratory tract infections in otherwise healthy toddlers in day care centres and the frequency of flu-like infections during the winter season in otherwise healthy elderly have been shown to be reduced by probiotics⁽¹¹⁾. The two requirements in the regulatory procedures, i.e. the demonstration of the causality of the probiotic effect on the reduction of disease risk and the extrapolation of the observed effects to the general population then become the hurdles for obtaining health claims. How to demonstrate that the components of the immune system that contribute to

protection against infection, e.g. a rhinovirus, are stimulated by probiotics? In future clinical studies, biomarkers of the immune system should be included, which then can be used as supporting evidence to demonstrate the causality of the beneficial effect⁽¹¹⁾. The choice of biomarkers or risk factors will depend on the specific clinical effect. For the aforementioned example of rhinovirus infection, these would include biomarkers of the acquired cellular immune system. It should be noted that from a scientific perspective the focus on underlying mechanisms of probiotic health effects is evident. From a regulatory perspective, the need for any demonstration of mechanisms could be questioned. Yet, from published opinions, it is clear that the NDA panel needs to be convinced of a cause and effect relationship between intake of probiotics and claimed health benefit.

Compared with other food and nutritional ingredients, demonstration of the mechanism of the probiotic effect is hampered by the biological complexity of this ingredient. In theory, the effect can be mediated by the complete organism or by specific components of the bacterium (e.g. CpG DNA, cell envelope or secreted proteins, capsular polysaccharides and metabolites). This sharply contrasts for instance with the immunomodulatory effects of a single molecule, such as vitamin D (see later).

Gut microbiota in relation to human health

A number of beneficial effects of probiotics dealing with gut health have been evaluated in Cochrane reviews. These meta-analyses have demonstrated the effect of probiotics on the prevention and treatment of antibiotic-associated diarrhoea⁽¹²⁾, necrotising enterocolitis⁽¹³⁾ and induction and maintenance of the remission time in pouchitis⁽¹⁴⁾. Another beneficial effect that has been demonstrated is prevention of traveller's diarrhoea⁽¹⁵⁾. Gut health also involves bowel function (transit time, frequency of bowel movements, quality of stools, etc), and improvement of bowel functions within the normal range is considered to be beneficial to human health.

Apart from traveller's diarrhoea, all of the studies mentioned earlier have been performed in individuals with a temporary or permanent impaired gut health. According to the EFSA criteria, patient studies may be used to substantiate health claims for the general population, but these are evaluated on a case-by-case basis. The exception is irritable bowel syndrome (IBS). According to the EFSA NDA panel, episodes of abdominal pain or discomfort occur both in healthy people and in individuals suffering from IBS, the difference being the higher frequency and greater severity of the symptoms in IBS. IBS patients are generally considered to be an appropriate study group to support claims on gastrointestinal discomfort intended for the general population. A similar case could be made for antibiotic-associated diarrhoea: in many cases for which antibiotics are prescribed, gut health *per se* is not affected or impaired. The unwanted side effect of antibiotics can be prevented by probiotics, which therefore can be considered as maintenance of gut health.

Digestive health requires a 'healthy' gut microbiota, but what is a healthy gut microbiota? Even if we take this to be

the gut microbiota of healthy individuals, the answer remains ambiguous. For the fifty-seven most common bacterial species identified by metagenome sequence analysis in the human gut, the inter-individual variability of abundance is between 12- and 2187-fold⁽¹⁶⁾. For less abundant bacteria, the inter-individual variation may be even greater. Moreover, the intestinal microbiota also changes in time as was illustrated recently in a study, in which age groups up to 100 years were compared⁽¹⁷⁾. Therefore, depending on the degree of differentiation of the technique, the composition of gut microbiota may be a unique individual characteristic, as has been shown previously⁽¹⁸⁾. However, a clearly distinct composition of gut microbiota, both compared with healthy individuals and between the two diseases, is found in inflammatory bowel disease (ulcerative colitis and Crohn's disease)⁽¹⁶⁾. Hence, it may be possible to distinguish an 'unhealthy' from a 'healthy' gut microbiota. While no direct health benefit can be attributed to a global increase in bifidobacteria, reducing specific pathogen carriage can be considered as beneficial. Gut microbiota biomarkers clearly need to be correlated with (and validated by) clinical endpoints.

The gut microbiota is of crucial importance for the correct development and function of the mucosal immune system. In a series of landmark publications^(7,19,20), Dennis Kasper's group has demonstrated that the capsular polysaccharide polysaccharide A of *Bacteroides fragilis* is indispensable for normal development of mucosal T lymphocytes and control of experimental colitis. In allergic diseases, the abnormalities in the composition of the gut microbiota precede the clinical expression of the disease⁽²¹⁾, although the cause and effect relationship has not been demonstrated yet. When evaluating totally of the evidence for the beneficial effects of probiotics on immune-mediated diseases, modulation of gut microbiota composition thus appears to be an important component. More research in human subjects, using state of the art microbiological methodologies, will be needed to confirm the role of probiotics in the improvement of gut microbiota equilibrium.

What can be learned from the scientific opinions of regulatory authorities thus far?

At this moment, there are more examples of negative opinions by the NDA of EFSA than positive ones, and the key factors for a successful health claim application of a given probiotic strain therefore cannot be extracted. Evaluation of all published opinions shows that applications can be rejected on the sole argument of insufficient characterisation of the product or the ingredient (e.g. the probiotic strain). A recently published EFSA opinion⁽²²⁾ lists a series of bacteria for which the health claims were dismissed on those grounds (see also the above section on Characterisation of probiotic bacteria). In many instances, the necessary information was in hand, but it was unclear that these data were requested in the dossier for submission. Whether approval of health claims under article 13.5, allowing for inclusion of proprietary data, also requires equally detailed strain identification is to be expected.

Health claims for (otherwise fully characterised) probiotic bacteria were further rejected because the submitted scientific

evidence is considered to be insufficient or irrelevant for the claim. The NDA panel wants to see the claimed health effect to be demonstrated in at least two randomised, (placebo) controlled clinical trials.

Important for the evaluation of a health claim is the cause and effect relationship. The approval of the health claim for vitamin D can be instructive in this respect. The EFSA panel has concluded that a cause and effect relationship has been established between the dietary intake of vitamin D and contribution to the normal function of the immune system and healthy inflammatory response⁽²³⁾. From the evidence submitted (in the form of three review papers^(24–26)), it appears that vitamin D has variable effects on different components of the immune system. Vitamin D enhances the killing of the intracellular micro-organisms *Mycobacterium tuberculosis* by monocytes because vitamin D induces cathelicidin, a potent antimicrobial protein⁽²⁴⁾. Vitamin D also inhibits the clearance of *Listeria monocytogenes*, which is another intracellular micro-organism⁽²⁵⁾. Vitamin D has been shown to inhibit a number of components of the adaptive immune system, including T lymphocyte proliferation and antibody production. These effects can be beneficial for reducing the incidence of autoimmune diseases but are unwanted in the case of infectious diseases.

The two strong points for vitamin D are that the compound is very well characterised, and that the receptors for vitamin D are expressed by cells of the immune system. The effects of the interaction between vitamin D and its receptor on lymphocytes/phagocytes are variable (sometimes stimulatory and sometimes inhibitory), but the conclusion that *vitamin D contributes to the normal function of the immune system* was accepted by the NDA panel. These strong points for vitamin D are impossible to reach for probiotics: bacteria are complex biological 'products', and extensive molecular characterisation will not change that. Receptors for probiotics could be at best for components of probiotics. For a number of strains, it has been demonstrated now that the probiotic bacteria can bind to receptors on cells of the immune system including dendritic cells⁽²⁷⁾. Therefore, emphasis on the physical interaction of probiotic bacteria with relevant receptors on cells of the immune system will be important to bring additional evidence on the underlying mechanisms of immunoregulatory effects of probiotics.

Conclusions and recommendations

Currently, there is a gap between the point where the biomedical science ends (with the publication of a paper in a scientific journal) and the point where the business begins (with the claim for a health benefit). Regulatory science that links the regulatory requirements of (in this case) probiotic product development to the science that ensures safety and functionality of probiotics will be needed to close this gap.

Because health claim approval requires both regulatory and basic sciences, both disciplines would benefit from a dialogue. One of the issues that needs to be discussed is the assessment of the quality of clinical studies, because many peer-reviewed studies are considered weak or even insufficient from the

regulatory point of view. However, clear-cut criteria defining gut health and immune health are lacking, and EFSA apparently works on a case-by-case scenario. For the future, it will be important to integrate the regulatory considerations in basic and clinical research. Characterisation of the probiotic product under study (including food matrix and background diet when appropriate) and selection criteria for the study population would be two obvious items to start with. Finally, a dialogue would be helpful to prevent that health claims for probiotics are lost in translation during the rewording of the conclusions from scientific research into a message that can be understood by the 'average consumer'.

The substantiation of the beneficial effects of probiotics into health claims that are approved by regulatory authorities and understood by the consumers is a joint responsibility of scientists, regulatory authorities, food and nutrition industry and consumers. An open dialogue and reaching consensus on a list of validated biomarkers for immune and gut health could be the first step in this process.

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References

1. European Parliament and the Council of the European Union (2007) Corrigendum to Regulation (EC) 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union **L 404**, 3–18. 30 December 2006. http://eur-lex.europa.eu/LexUriServ/site/en/oj/2007/L_012/L_01220070118en00030018.pdf
2. Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria (2001) Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. FAO Food and Nutrition paper 85, 1–50.
3. Guidance on the implementation of regulation N°1924/2006 on nutrition and health claims made on Foods. Conclusions of the standing committee on the food chain and animal health. *Ernährung/Nutrition* **31**, 468–472. http://ec.europa.eu/food/food/labellingnutrition/claims/guidance_claim_14-12-07.pdf
4. Rijkers GT, Bengmark S, Enck P, *et al.* (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. *J Nutr* **140**, 671S–676S.
5. Cummings JH, Antoine JM, Azpiroz F, *et al.* (2004) PASS-CLAIM—gut health and immunity. *Eur J Nutr* **43**, Suppl. 2, II118–II173.

6. Azaïs-Braesco V, Bresson JL, Guarner F, *et al.* (2010) Not all lactic acid bacteria are probiotics, ... but some are. *Br J Nutr* **103**, 1079–1081.
7. Mazmanian SK, Round JL & Kasper DL (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* **453**, 620–625.
8. Kankainen M, Paulin L, Tynkkynen S, *et al.* (2009) Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proc Natl Acad Sci U S A* **106**, 17193–17198.
9. World Health Organization (1992) *Basic Documents*, 39th ed. Geneva: WHO.
10. Albers R, Antoine JM, Bourdet-Sicard R, *et al.* (2005) Markers to measure immunomodulation in human nutrition intervention studies. *Br J Nutr* **94**, 452–481.
11. Wolvers D, Antoine JM, Myllyluoma E, *et al.* (2010) Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of infections by probiotics. *J Nutr* **140**, 698S–712S.
12. Johnston BC, Supina AL, Ospina M, *et al.* (2007) Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *The Cochrane Database of Systematic Review* issue 2, CD004827.
13. Guthmann F, Kluthe C & Bühner C (2010) Probiotics for prevention of necrotising enterocolitis: an updated meta-analysis. *Klin Padiatr* **222**, 284–290.
14. Sandborn WJ, McLeod RS & Jewell DP (1998) Pharmacotherapy for induction and maintenance of remission in pouchitis. *The Cochrane Database of Systematic Review* issue 2, CD001176.
15. McFarland LV (2007) Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Med Infect Dis* **5**, 97–105.
16. Qin J, Li R, Raes J, *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65.
17. Biagi E, Nylund L, Candela M, *et al.* (2010) Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* **5**, e10667.
18. Zoetendal EG, Rajilic-Stojanovic M & de Vos WM (2008) High-throughput diversity and functionality analysis of the gastrointestinal tract microbiota. *Gut* **11**, 1605–1615.
19. Duan J, Chung H, Troy E, *et al.* (2010) Microbial colonization drives expansion of IL-1 receptor 1-expressing and IL-17-producing gamma/delta T cells. *Cell Host Microbe* **7**, 140–150.
20. Avci FY & Kasper DL (2010) How bacterial carbohydrates influence the adaptive immune system. *Annu Rev Immunol* **28**, 107–130.
21. Sjögren YM, Jenmalm MC, Böttcher MF, *et al.* (2009) Altered early infant gut microbiota in children developing allergy up to 5 years of age. *Clin Exp Allergy* **39**, 518–526.
22. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) Scientific opinion on the substantiation of health claims related to non-characterised bacteria and yeasts pursuant to Article 13(1) of regulation (EC) No 1924/2006. *EFSA J* **8**, 1470.
23. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) Scientific opinion on the substantiation of health claims related to vitamin D and normal function of the immune system and inflammatory response (ID 154, 159), maintenance of normal muscle function (ID 155) and maintenance of normal cardiovascular function (ID 159) pursuant to Article 13(1) of regulation (EC) No 1924/2006. *EFSA J* **8**, 1468.
24. Bikle D (2009) Nonclassic actions of vitamin D. *J Clin Endocrinol Metab* **94**, 26–34.
25. Cantorna MT, Yu S & Bruce D (2008) The paradoxical effects of vitamin D on type 1 mediated immunity. *Mol Aspects Med* **29**, 369–375.
26. Khazai N, Judd SE & Tangpricha V (2008) Calcium and vitamin D: skeletal and extraskelatal health. *Curr Rheumatol Rep* **10**, 110–117.
27. Lebeer S, Vanderleyden J & De Keersmaecker SC (2010) Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat Rev Microbiol* **8**, 171–184.