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Review article

Should yoghurt cultures be considered probiotic?

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Probiotics are live micro-organisms that when administered in adequate amounts confer a health benefit on the host. Consumption of yoghurt has been shown to induce measurable health benefits linked to the presence of live bacteria. A number of human studies have clearly demonstrated that yoghurt containing viable bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*) improves lactose digestion and eliminates symptoms of lactose intolerance. Thus, these cultures clearly fulfil the current concept of probiotics.

Bacteria: Functional Food: Probiotic definition: Market regulation

Fermented milks and yoghurts have been used throughout the history of mankind. A proposal by the European Commission for a council regulation laying down additional rules on the common organization of the market in milk and milk products for yoghurt and yoghurt-like products was presented recently (AGRI/38 743/2003rev3). Article 2 and Annex of the proposal establish that 'yoghurt' is a product obtained by the fermentation of milk with cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*. The term 'yoghurt-like product' is defined as alternative culture yoghurt (i.e. when *L. bulgaricus* is substituted by other *Lactobacillus* species for the fermentation of milk) or yoghurt containing probiotic bacteria (when probiotic bacteria are added to the yoghurt or alternative cultures). Article 4 of the proposal declares that 'probiotic bacteria' are live food supplements, which benefit the health of the consumer.

These statements and definitions seem to fit well with the common knowledge and use of the terms by scientists, industry and the general public. The proposal clearly differentiates between yoghurt starter cultures (*S. thermophilus* and *L. bulgaricus*) and probiotic bacteria that may be added (see Article 2 and Annex), but the distinction does not exclude *S. thermophilus* and *L. bulgaricus* from the category of probiotic bacteria. The starter cultures can also be probiotics as long as they comply with the definition of probiotic. For instance, if some of the health benefits achieved by consumption of yoghurt are linked to the presence of live bacteria, then these bacteria are probiotics (defined by the proposal as 'live food supplements which benefit health').

The purpose of the present article is to produce a critical review on the notion of probiotics, and to analyse whether the usual yoghurt cultures (*S. thermophilus* and *L. delbrueckii* sp. *bulgaricus*) qualify for this notion according to our current concepts. If the starter cultures are probiotics, products that have been heat-treated after fermentation would lack the probiotic benefit of yoghurt, even if other nutritional properties are unchanged by heat treatment. As mentioned by the council regulation (see Point 9 in the Preamble), legislation of most EU member states establishes that the presence of living bacteria in large quantities is a characteristic of yoghurt, and the notion of yoghurt should therefore be reserved to this kind of product. In the present article, the term 'yoghurt' refers to the fermented milk product in which the starter micro-organisms are viable, live and abundant (Codex Standard for Fermented Milks, 243-2003).

Probiotics: historical evolution of the concept

The origin of the term 'probiotic' is credited to Werner Kollath as related in a publication by the German scientist Ferdinand Vergin

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(1954). Kollath proposed the term 'Probiotika' to designate 'active substances that are essential for a healthy development of life'. The Greek meaning of the term ('for life') is opposed to 'antibiotics' ('against life'), at that time a very well recognized 'hit' of science. In a paper published in *Science* a few years later, Lilly & Stillwell (1965) described probiotics as substances secreted by one micro-organism that stimulate the growth of another. Other US scientists later used the term probiotic with the same meaning: factors that stimulate growth (Sperti, 1971; Nutini *et al.* 1982). However, Parker (1974) made a different use of the term, which was applied to describe animal feed supplements specifically designed to improve health. He introduced a new definition: 'organisms and substances which contribute to intestinal microbial balance'. The success of the new concept is mainly due to the subsequent work of Roy Fuller in Reading (UK), who revised Parker's definition by removing the reference to 'substances'. Thus, a probiotic is 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Fuller, 1989). The concept was also applicable to human nutrition and medicine (Fuller, 1991). This definition stressed the importance of viable microbial cells as an essential requirement, but kept the concept restricted to a particular mechanism of action: improvement of the intestinal microbial balance, as in Parker's definition. Shortly thereafter, Havenaar & Huis In't Veld (1992) broadened the definition to include the microflora of other habitats different from the intestinal, such as the upper respiratory tract or the urogenital tract. Probiotics are 'a mono- or mixed culture of live microorganisms which applied to animal or man, affects beneficially the host by improving the properties of the indigenous microflora'. Remarkably, the concept was still restricted to micro-organisms able to influence the indigenous microbial balance.

The notion of probiotic as introduced by Fuller was attractive and successful. Subsequent scientific approaches aimed at the identification of the ideal probiotic, and discussed the characteristics and properties required for a micro-organism to qualify as a probiotic. Many scientific publications and reviews listed a series of essential requirements to be checked by *in vitro* methods in the screening of micro-organisms with a probiotic value. It was suggested that only strains shown to possess these essential traits should be tested *in vivo*. The list of essential requirements based on theoretical considerations included the following:

(1) Human origin (as a token of safety for human use);
(2) Resistance to gastric acidity and bile toxicity (these properties would predict good survival during gastrointestinal transit);
(3) Adhesion to gut epithelial cells (as a requirement for successful colonization *in vivo* – the term colonization describes the ability of a particular bacteria strain to permanently establish in the host over time without the need for periodic reintroduction of the bacteria; see Bezkorovainy, 2001);
(4) Production of antimicrobial substances or bacteriocins (for pathogen antagonism);
(5) Ability to modulate immune responses and ability to influence metabolic activities of faeces (for prevention of colon cancer).

Several bacteria strains successfully qualified by passing through all the *in vitro* tests, and thus received the 'full title' of being a probiotic, in some cases without any proof of a beneficial health effect demonstrated in human studies.

On the other hand, most recent scientific developments have challenged the validity and usefulness of the suggested criteria for a full definition of probiotic. For instance, the Nissle *Escherichia coli* strain, isolated in 1917 for therapeutic purposes in the pre-antibiotic era, is not resistant to acid or bile toxicity. This strain is given in enteric-coated capsules and has proved useful for the prevention and treatment of human disease in well-designed human studies (see for instance the *Lancet* paper on a clinical trial in ulcerative colitis; Rembacken *et al.* 1999). There is no proof so far that supports or substantiates the claim linking human origin and safety for human use, or human origin and efficiency in human studies. It is also well known that many pathogens exert their deleterious effect through adhesion to gut epithelial cells (Hoepelman & Tuomanen, 1992), and again this fact has cast some doubts about the meaning of this property by itself in the definition of a strain as probiotic (Ducluzeau, 2002).

Taken together, these observations suggested that the proposed list of *in vitro* properties could no longer be accepted as criteria for definition of a probiotic. Most common views about the *in vitro* tests for probiotics among the scientific community are well reflected in the report by the Joint Food and Agriculture Organization/World Health Organization Working Group (2002):

> 'In vitro tests are useful to gain knowledge of strains and the mechanism of the probiotic effect. However, it was noted that the currently available tests are not fully adequate to predict the functionality of probiotic microorganisms in the human body. It was also noted that in vitro data available for particular strains are not sufficient for describing them as probiotic. Probiotics for human use will require substantiation of efficacy with human trials.'

Hence, *in vitro* studies are and will be a very useful tool for the selection of bacteria for a particular probiotic use, but are not essential requirements for a strain to qualify as a probiotic. In addition to this consensus about the *in vitro* tests, some important evidence obtained in human studies has challenged Fuller's concept of probiotics. First, many bacteria able to transit alive through the entire human gastrointestinal tract are devoid of a measurable health effect, and second, a persistent change in the indigenous flora by consumption of a probiotic has never been demonstrated (Bezkorovainy, 2001). According to these observations, induction of changes in the indigenous flora should not be considered as a primary target of probiotics. Thus, newer definitions of the probiotic concept have omitted the need to induce changes in the microbial balance, as health benefits can be produced through other mechanisms as well.

The current concept of probiotics

Definitions proposed in recent years are listed below.

(1) 'Oral probiotics are living micro-organisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition'. LABIP consensus definition (Guarner & Schaafsma, 1998).
(2) 'A live microbial food ingredient that is beneficial to health'. Proposed by Salminen *et al.* (1998) and adopted as consensus definition by the FUFOSE Concerted Action sponsored by the European Commission (Diplock *et al.* 1999).
(3) 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Definition by the Joint Food and Agriculture Organization/World Health

Organization Working Group (2002). The International Scientific Association for Probiotics and Prebiotics recently adopted this definition (Reid *et al.* 2003).

(4) 'Probiotic bacteria are live food supplements which benefit the health of the consumer', as defined in the legal proposal by the European Commission referred to earlier.

All these definitions require that the term probiotic should only be applied to microbes administered alive having a demonstrated beneficial effect (Reid *et al.* 2003). The similarities between these new definitions clearly reflect the consensus of scientists all over the world on this issue. The concept is now open to many different applications in a large variety of fields relevant for human and animal health. The concept is generic and covers many different aspects that may be addressed by specific strains.

Yoghurt cultures are probiotic

According to current scientific concepts, yoghurt cultures are probiotics if a beneficial physiological effect can be obtained by consumption of the live cultures and the benefit has been substantiated appropriately in human studies.

All *S. thermophilus* and most *L. bulgaricus* strains have a high lactase activity (Sanders *et al.* 1996). It is well recognized that yoghurt consumption improves lactose digestion and eliminates symptoms of lactose intolerance. The physiological effects have been clearly demonstrated in a large number of human studies in which consumption of yoghurt (with live cultures) has been compared with consumption of a pasteurized product (with heat-killed bacteria; Gilliland & Kim, 1984; Savaiano *et al.* 1984; McDonough *et al.* 1987; Dewit *et al.* 1988; Lerebours *et al.* 1989; Pochart *et al.* 1989; Marteau *et al.* 1990; Varela-Moreiras *et al.* 1992; Rizkalla *et al.* 2000; Labayen *et al.* 2001; Pelletier *et al.* 2001). All studies have shown better lactose digestion and absorption in subjects who consumed yoghurt with live cultures, as well as reduction of gastrointestinal symptoms. The benefit on lactose absorption was also demonstrated in healthy subjects without lactose maldigestion (Rizkalla *et al.* 2000). All these studies highlight the essentiality of live bacteria for the beneficial effect on lactose digestion (not excluding that other beneficial effects can be due to non-viable bacteria). There are no major scientific discrepancies on this issue fully established by human intervention studies.

The functional properties of yoghurt are consistent with further evidence obtained in important ancillary studies that confirmed viability and metabolic activity of yoghurt bacteria in the human intestine (Martini *et al.* 1987; Pochart *et al.* 1989; Marteau *et al.* 1990), as well as in *in vivo* animal models (Lick *et al.* 2001; Drouault *et al.* 2002). Yoghurt bacteria can also be detected in faeces of human subjects consuming yoghurt (Brigidi *et al.* 2003; Callegari *et al.* 2004).

Yoghurt is also being used in the management of acute diarrhoeal disorders, as recommended by World Health Organization (1995). This recommendation is based on the traditional approach in many countries all over the world, as well as on evidence gained in human intervention studies (Boudraa *et al.* 1990, 2001). Yoghurt feeding in children with acute watery diarrhoea decreased stool frequency and shortened the duration of diarrhoeal episodes (Boudraa *et al.* 2001).

Other studies have addressed the role of yoghurt on the immune system. Yoghurt consumption may enhance the immune response particularly in immunocompromised populations, such as the elderly (Meydani & Ha, 2000). The role of yoghurt in the modulation of the immune system was further demonstrated by Van de Water *et al.* (1999) in a randomized controlled trial with human subjects. Long-term consumption of yoghurt, as compared with either the same product heat-treated after fermentation or exclusion of yoghurt products from the diet during the length of the study (1 year), was associated with a significant decrease in allergic symptoms.

In agreement with the demonstration of probiotic efficacy, several consensus documents have acknowledged the probiotic nature of yoghurt cultures. These include the report of the Joint Food and Agriculture Organization/World Health Organization Working Group (2002), the International Scientific Association for Probiotics and Prebiotics workshop consensus document (Reid *et al.* 2003), the *Lancet* review on gut flora in health and disease (Guarner & Malagelada, 2003) and the official web page of the Ministry of Agri-culture and Agri-Food in Canada (http://www.agr.gc.ca/food/nff/FAQs.html#categories).

Conclusions

The concept of 'probiotic' has evolved to a simple and straightforward notion: probiotics are 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host'. Consumption of yoghurt has been shown to induce measurable health benefits linked to the presence of live bacteria, as compared with products with heat-killed bacteria. Thus, yoghurt starter cultures clearly fulfil the current concept of probiotics at least for its beneficial effect on lactose digestion *in vivo*. Some yoghurt cultures were shown to induce other health benefits such as reduction in severity and duration of acute diarrhoea, or prevention of allergic disorders. Whether all yoghurt cultures can lead to these health benefits may require further substantiation.

Conflict of interest

F. G. is a member of the Beneo Scientific Committee sponsored by Orafti (Tienen, Belgium). L. M. served as coordinator of a research project 'Detection in faecal samples of yoghurt cultures', which was co-funded by the Groupe Danone (Paris, France). B. K. serves as the coordinator of a multi-centre research project 'Childhood Obesity – Programming by Infant Nutrition', funded by the European Commission (QLK1-2001-00 389) and co-funded by the Groupe Danone (Paris, France). S. S. is holder of the Bristol-Myers Squibb Foundation Unrestricted Nutrition Research Grant.

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