Journal of Biotechnology 162 (2012) 356–365

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Science and technology for the mastership of probiotic applications in food products

K. Makinen*, B. Berger, R. Bel-Rhlid, E. Ananta

Nestlé Research Center, Nestec Ltd. Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland

ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 10 July 2012
Accepted 13 July 2012
Available online 22 July 2012

Keywords:
Probiotic
Health benefit
Health claim
Manufacturing
Stability
Storage
Biotransformation

ABSTRACT

Probiotics, defined as live microorganisms which when consumed in adequate amounts confer a health benefit on the host, are a common part of our daily diet. Since their conception in the early 20th century, the health benefit applications of probiotics have been expanding, culminating in the recent challenge of health claim substantiation in Europe. This paper highlights the different application areas of probiotics, introduces the use of non-viable microorganisms to confer health benefits, and explains the recent regulatory challenges surrounding probiotics. It then describes in detail the different stages in the development of food products containing probiotic bacteria starting from the selection of suitable strains for industrial production. The description of production of probiotic powders with specific focus on strategies to maintain high viability during drying and storage then follows. The paper finishes with a discussion of probiotic stability in liquid products, followed by a description of the use of probiotics to improve nutrient bioavailability and digestibility of the food products, which they ferment or biotransform.

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1. History of probiotics

Fermentation is one of the oldest methods of preserving foods. By 6000 BC, cheese was being made from cow's and goat's milk in China. Fermented products such as kefir, koumiss, leben, and dahi were also used therapeutically long before the existence of microorganisms was discovered by Leeuwenhoek in 1683. Louis Pasteur isolated lactic acid bacteria from milk in 1857, but it was in 1907 that the concept of probiotics was born through Elie Metchnikoff's postulation such that consumption of fermented foods led to the prolongation of life. He based his hypothesis on the observation that nomads in Bulgaria and the Russian Steppes who consumed large amounts of fermented milk appeared to live exceptionally long. Metchnikoff hypothesized that proteolytic bacteria in the gut such as Clostridia produced toxic substances such as indoles, phenols, and ammonia, which led to autointoxication. This, in turn, could be countered by consumption of fermented food that contained harmless lactic acid bacteria, which suppressed the growth of proteolytic bacteria by lowering the intestinal pH (Metchnikoff, 1907). Although Metchnikoff's postulate was subsequently disputed (Cheplin and Rettger, 1920), it started a chain of events that culminated in the coining of the term "probiotic" by Kollath in 1953, which he defined as "active substances that are essential for a healthy development of life" (Hamilton-Miller et al., 2003).

2. The definition of probiotics

Today, no legal definition of "probiotics" exists, but the definition accepted by most is the definition of the 2001 joint WHO/FAO expert consultation: "probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002, 2001). We now know that some microorganisms that have been inactivated prior to consumption or extracts of microorganisms can also be health-beneficial (Adams, 2010), but for the time being, they are disqualified from the genre of probiotics. In contrast, a glance at the market place reveals that some strains or combinations of strains are marketed as being "probiotic" without a shred of evidence of their efficacy in humans. This is misuse of the term "probiotic", which implies a demonstrated (and not only purported) health benefit. Such misuse of the term is likely to become less frequent, however, as the regulatory environment of Europe and elsewhere becomes increasingly stringent, thereby limiting the use of the term probiotic to those strains with appropriate scientific backing. Another misuse of the term probiotic is typified by a "probiotic mattress" (available on the market at the time of this writing) incorporating a layer of microorganisms to counter dust mite allergens. Because in this case, the microorganism is not administered to the host, but rather, resides in the vicinity of the host, the term probiotic should not be used. Overall, 11 years

* Corresponding author. Tel.: +41 21 7858740; fax: +41 21 7859486.
E-mail address: kimmo.makinen@rdls.nestle.com (K. Makinen).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.07.006

after its conception, the WHO/FAO definition of probiotics remains sufficiently accurate, except that in the light of current knowledge, the inclusion of non-live microorganisms in the definition seems justified (see below).

3. Health benefit application areas of probiotics

To date, over 900 human intervention studies and countless reviews have been published on the health beneficial effects of probiotics. The studies vary widely in quality and have been conducted with a range of probiotic strains, health benefits, and target populations. The health benefits of probiotics have been reviewed extensively elsewhere (Deshpande et al., 2011; Rowland et al., 2010) and will not be detailed here. It is noteworthy, however, that the health beneficial properties of probiotics are strain-specific. This means that, for example, two *Lactobacillus acidophilus* strains, however closely related, may not be presumed to have the same health beneficial properties unless so proven in clinical trials. A case in point is the scientific opinion from the European Food Safety Authority (EFSA) on *Lactobacillus johnsonii* BFE 6128, in which all the evidence provided by the applicant was dismissed for being irrelevant as it pertained to another related *L. johnsonii* strain, *L. johnsonii* La1, rather than the strain under application (EFSA NDA panel, 2011). It is also important not to assume that a probiotic strain shown to be health beneficial when administered alone, has the same benefit when administered in combination with other strains. Therefore, any novel mixture of probiotic strains, even if it contains a well-studied probiotic strain, should be substantiated in a separate set of studies.

The health benefit areas accounting for the biggest market share of probiotic sales are immune protection and gut comfort in the general healthy adult population (Euromonitor International, 2010; Mellentin, 2008). Their success to some extent reflects the level of existing scientific evidence, but is greatly aided by the high consumer understanding of, and demand for these health benefits. New innovate applications of probiotics are also emerging. For example, some evidence suggests that *L. johnsonii* La1 consumed orally may expedite the recovery of skin immune functions in healthy subjects following UV exposure (Peguet-Navarro et al., 2008). *L. reuteri* DSM17938 has, in turn, been shown to significantly reduce crying time in colicky babies, possibly by improving gastric emptying (Savino et al., 2010, 2007). Interestingly, a combination of *L. helveticus* R0052 and *B. longum* R0175 has even been shown to reduce anxiety-related symptoms in the general population (Messaoudi et al., 2011) through a mechanism that may involve stimulation of the parasympathetic nervous system (Bravo et al., 2011). Finally, studies are emerging showing a positive effect of probiotics on body weight in healthy overweight adults (Kadooka et al., 2010) and cholesterol levels in hypercholesterolemic adults (Jones et al., 2011). Therefore, the potential applications of probiotics are many. The major challenge is often to identify which bacterial strain will be most likely to be successful for a given health benefit.

4. Identification and development of probiotics

Traditionally, and reinforced by the WHO/FAO guidance document, probiotic candidate strains have been selected on the basis of a few simple properties. These are survival in simulated gastrointestinal conditions (incubation at pH 2.5 followed by incubation in the presence of bile salts), the ability to adhere to intestinal epithelial cells, and the production of antimicrobial substances. We now know, however, that these properties neither predict health benefits in humans, nor are they *sine qua non* conditions for the strain to be health beneficial. For example, experiments show that *Bifidobacteria* sp. are highly sensitive to adult gastric acidity while *Lactobacilli* are relatively tolerant to it (C. Cavadini, unpublished observations). Also, even though it is conceptually attractive that a probiotic strain should be able adhere to intestinal epithelium, systematic investigation of the importance of this characteristic in humans is lacking. Finally, the production of antimicrobial substances may only be relevant in cases where a specific antipathogenic effect is desired. Nevertheless, such selection criteria have found their way to some recent probiotic regulatory guidelines, for example those adopted in India in 2011 (ICMR-DBT, 2011). Instead of, or in addition to these basic "probiotic properties", candidate probiotic strains are typically selected via a process involving a series of *in vitro* and pre-clinical tests. They can include, but are not limited to, assays testing the ability of the probiotic strain to modulate immune cell function *in vitro* or *in vivo*, which can give an indication of what immune profile the probiotic strain might induce upon interaction with the immune system in humans. Nevertheless, the final health benefit must always be demonstrated in well-designed human trials. To increase the likelihood of success in human intervention trials, which are costly, better predictive *in vitro* and pre-clinical systems and a better understanding of the mechanism of action of probiotics are needed.

For certain health benefits, probiotics can be developed using a 'pharma style' approach, which relies on identification of a "drugable" target and selection of probiotic strains that can influence it. For example, *L. reuteri* NCIMB 30242 (Cardioviva™), was developed as a cholesterol-lowering probiotic based on its high level of expression of a class of enzymes called bile salt hydrolazes (BSH). BSH deconjugate bile acids in the intestine, reducing their reabsorption and forcing more cholesterol to be shunted into resynthesis of bile. In a human clinical trial, the strain was shown to reduce LDL-C in hypercholesterolemic subjects by nearly 9%, over 6 weeks, which is comparable to levels achieved by plant sterols (Jones et al., 2011). Such 'pharma-style' identification and development of candidate probiotic strains is likely to increase.

5. The use of non-viable probiotics

Even though the use of the term "probiotic" is currently limited to microorganisms alive at the time of consumption, it is becoming increasingly evident that even non-viable microorganisms can confer health benefits (Adams, 2010). In fact, products containing non-viable microorganisms have been available on the market since 1907 when Pierre Boucard isolated two strains of Lactobacilli from human stool, heat-killed them, and marketed them as an anti-diarrhea supplement called Lacteol™. The anti-diarrhea benefit was later confirmed in clinical studies (Salazar-Lindo et al., 2007) and thus, Lacteol™ is still available as over-the-counter medication in a number of countries. In addition to diarrhea treatment, heat-killed microorganisms have been shown to be effective against allergic conditions in children (Morisset et al., 2011; Peng and Hsu, 2005) and adults (Ishida et al., 2005), with more research likely to become published in other health benefit areas soon.

In addition to heat-treatment, inactivation of microorganisms can be achieved through sonication, high pressure treatment, freeze-thawing, or irradiation (not permitted for food applications). However, in the published studies, heat-treatment has been the method of choice for strains consumed in foods. Bacterial lysates produced by sonication have, in turn, been investigated in the context of topical applications for skin health benefits, such as treatment of atopic dermatitis (Di et al., 2003; Gueniche et al., 2008).

It should also be noted that even though non-viable microorganisms can be produced deliberately, any probiotic product will contain many non-viable microorganisms in addition to the live ones, due to the inevitable loss of viability during manufacture and

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