Chapter 10
Latest Developments in Probiotics

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Introduction

Probiotic foods are a group of health-promoting, so-called functional foods, with large commercial interest and growing market shares (Arvanitoyannis & van Houwelingen-Koukaliaroglou, 2005). In general, their health benefits are based on the presence of selected strains of lactic acid bacteria (LAB), that, when taken up in adequate amounts, confer a health benefit on the host. They are administered mostly through the consumption of fermented milks or yoghurts (Mercenier, Pavan, & Pot, 2003). In addition to their common use in the dairy industry, probiotic LAB strains may be used in other food products too, including fermented meats (Hammes & Hertel, 1998; Incze, 1998; Kröckel, 2006; Työppönen, Petäjä, & Mattila-Sandholm, 2003). Although the concept is not new, only a few manufacturers offer fermented sausages with probiotic LAB. This is probably due to the more artisan orientation of sausage manufacturers as compared to the dairy industry, a larger variety of products, as well as a number of uncertainties concerning technological, microbiological, and regulatory aspects (Kröckel, 2006). The application of probiotic LAB must in all cases be based on a careful selection procedure, if any health claims are to be taken into account. The present chapter gives an overview of research activities that have previously explored the potential of probiotic LAB strains in fermented meats and aims at giving a critical interpretation of the results obtained.

Probiotics

History and Definitions

Although probiotics are usually linked with gut health, the first suggestion of a beneficial association between microorganisms and the human host can probably

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be attributed to Albert Döderlein, who proposed in 1892 that lactic acid production by vaginal bacteria prevented or inhibited the growth of pathogenic bacteria (Döderlein, 1892). Most cited as the founding father of the probiotic concept, however, is Ilya Metchnikoff. In his work, “The Prolongation of Life – Optimistic Studies”, published in 1908, he implicated a lactic acid bacterium found in Bulgarian yoghurts as the agent responsible for deterring intestinal putrefaction and ageing (Metchnikoff, 1908). Hence, he became the first to speculate on the potential health-promoting and even life-lengthening properties of LAB. Another milestone in the history of probiotics is undoubtedly the work of Minoru Shirota, who was the first to actually cultivate a beneficial intestinal bacterium, *Lactobacillus casei* Shirota, and distribute it in a dairy drink that was introduced to the market in 1935 (Yakult Central Institute for Microbiological Research, 1999).

The word “probiotic” stems from the Greek πρo βιος (pro bios, “for life”) and was originally proposed to describe growth-promoting substances produced by one protozoan for the benefit of another (Lilly & Stillwell, 1965). In 1974, it was first linked with the intestinal microbial balance by Parker (1974) in his work on animal feed supplements with beneficial effect on the host. In the following decades, the concept of probiotics was regularly defined and redefined. Nowadays, a widely accepted definition is a rather broad one that was proposed by a Joint Expert Consultation of the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO). It classifies probiotics as “life microogranisms that, when consumed in an adequate amount, confer a health benefit on the host” (FAO/WHO, 2001). This definition does not emphasize on the nature of the host (animal or human), the origin of the microorganisms (human or non-human), or the ability to adhere to body surfaces. Also, although stressed elsewhere in the FAO/WHO report, the viability at the target site is not considered a restriction, thus leaving the door open for the acceptance of the yoghurt bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as probiotic microorganisms as well as of the health effects ascribed to certain cell components. As no site of action is precise, probiotic preparations can not only be targeted towards the benefit of the gut, but, for instance, also towards that of the oral cavity, the nasopharynx, the respiratory tract, the stomach, the vagina, the bladder, and the skin (Reid, 2005). Of course, no definition is final, and, amongst others, the viability of the probiotics, or better, their effectiveness after food processing and storage, their site of activity, the numbers necessary to exert a beneficial effect, the format of intake, and the nature of the carrier remain critical issues in a vivid discussion (Makras, Avonts, & De Vuyst, 2004; Mercenier et al., 2003; Senok, Ismaeel, & Botta, 2005).

As probiotics are mainly directed to alter the composition and/or metabolic activity of the gut microbiota towards what is generally believed to be a healthy or balanced one, that is, being predominantly saccharolytic and comprising significant numbers of bifidobacteria and lactobacilli (Picard et al., 2005), pre- and synbiotics need to be mentioned as alternative or complementary strategies to achieve this goal. Prebiotics are selectively fermented, non-digestible, food ingredients that allow specific changes, both in the composition and/or activity of the gastrointestinal microbiota, that confer benefits upon host well-being and health (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004; Gibson & Roberfroid, 1995). To this day, sufficient scientific evidence exists to recognise three types of carbohydrates as prebiotics, namely inulin-type fructans, transgalacto-oligosaccharides, and lactulose (Gibson et al., 2004), although the latter should be considered as a laxative drug (Bass & Dennis, 1981). For some other candidate prebiotics, promising data have been published but further investigation is required (Gibson et al., 2004). Finally, synbiotics are mixtures of pro- and prebiotics, wherein, the latter is thought to improve survival and implantation of the former, either by stimulating growth or by metabolically activating the health-promoting bacteria, thereby taking advantage of the individual and possibly the synergistic health effects of both components (Gibson & Roberfroid, 1995; Rastall & Maitin, 2002).

Probiotic Microorganisms

Different types of food products or food supplements containing viable probiotic microorganisms with health-promoting properties are commercially available, either as fermented or non-fermented food commodities, or as specific food supplements and pharmaceutical preparations in the form of powders, tablets, or capsules. The first step in the development of such products is the selection of an appropriate microbial strain. Throughout the years, several selection criteria for probiotics have been formulated, including human origin, non-pathogenic behaviour, safety, resistance to gastric acidity and bile toxicity, adhesion to or interaction with the gut epithelial tissue, ability to persist within the gastrointestinal tract, production of antimicrobial substances and nutraceuticals, evidence of beneficial health effects, ability to influence metabolic activities, and resistance to technological processes (Dunne et al., 2001; Maldonado Galdeano, de Moreno de LeBlanc, Vinderola, Bibas Bonet, & Perdigón, 2007; Ross, Desmond, Fitzgerald, & Stanton, 2005). Although some of the present criteria appear obvious, others are directly related with the definition of probiotics handled by the authors. It should, however, be stressed that characteristics such as survival of the passage through the upper gastrointestinal tract, thus resisting the action of gastric juice, bile salts, and proteolytic enzymes, are not sufficient to call a certain microbial strain as probiotic. According to the WHO/FAO definition, the main criterion for a probiotic strain should be the fact that it confers a health benefit on the host (FAO/WHO, 2001). This benefit can only be demonstrated through well-designed, randomized, double blind, placebo-controlled, multi-centre human trials, the results of which are published in peer-reviewed international scientific journals (Guarner & Schaafsma, 1998; Salminen et al., 1998). Furthermore, it should be stressed that probiotic effects are strain-dependent, and extrapolation of the existing data from closely related microorganisms is not sufficient to identify a strain as probiotic (Mercenier et al., 2003). Exact identification and characterization of the potential probiotic strain used at the genus, species, and even strain level, using internationally accepted methodologies, should be the

Table 10.1 Microorganisms whose strains are used or considered for use as probiotics [adapted from (Collins & Gibson, 1999; Makras, 2004; Senok et al., 2005)]

Lactobacillus sp.	*Bifidobacterium* sp.	Other Lactic Acid Bacteria	Other microorganisms
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis* [a]	*Bacillus cereus* [a,b]
L. amylovorus	*B. animalis* subsp. *animalis*	*Enterococcus faecium* [a]	*Bacillus subtilis* [b]
L. brevis	*B. animalis* subsp. *lactis*	*Lactococcus lactis*	*Clostridium butyricum*
L. casei	*B. bifidum*	*Leuconostoc mesenteroides*	*Escherichia coli* [b]
L. crispatus	*B. breve*	*Sporolactobacillus inulinus* [a]	*Propionibacterium freudenreichii* [a,b]
L. curvatus	*B. longum*	*Streptococcus thermophilus*	*Saccharomyces cerevisiae* [b]
L. delbrueckii subsp. *bulgaricus*			*Saccharomyces boulardii* [b]
L. fermentum			
L. gallinarum[a]			
L. gasseri			
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			
L. salivarius			

[a] mainly applied in animals
[b] mainly applied in pharmaceutical preparations

first step in every process of development of probiotic food product (Reid, 2005). It is generally believed that rational selection of probiotics will be facilitated by the recent availability of genome sequences of some probiotic and candidate probiotic strains, allowing the prediction of their physiological profiles (Klaenhammer, Barrangou, Buck, Azcarate-Peril, & Altermann, 2005; Leahy, Higgins, Fitzgerald, & van Sinderen, 2005).

Up to now, mainly bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* have been used or considered as probiotics, besides other bacteria (mostly belonging to the group of LAB) and some yeasts (Table 10.1).

Health Benefits: Prophylactic and Therapeutic Effects of Probiotics

A wide variety of potential beneficial health effects have been attributed to probiotics (Table 10.2). Claimed effects range from the alleviation of constipation to the prevention of major life-threatening diseases such as inflammatory bowel disease, cancer, and cardiovascular incidents. Some of these claims, such as the effects of probiotics on the shortening of intestinal transit time or the relief from

Table 10.2 Potential and established health benefits associated with the usage of probiotics [adapted from (FAO/WHO, 2001; Mercenier et al., 2003; Naidu, Bidlack, & Clemens, 1999; Parvez, Malik, Ah Kang, & Kim, 2006; Sanders, 1998; Sanders, & Huis in ’t Veld, 1999)]

Health benefit	Proposed mechanism(s)
Cancer prevention	Inhibition of the transformation of pro-carcinogens into active carcinogens, binding/inactivation of mutagenic compounds, production of anti-mutagenic compounds, suppression of growth of pro-carcinogenic bacteria, reduction of the absorption of carcinogens, enhancment of immune function, influence on bile salt concentrations
Control of irritable bowel syndrome	Modulation of gut microbiota, reduction of intestinal gas production
Management and prevention of atopic diseases	Modulation of immune response
Management of inflammatory bowel diseases (Crohn’s disease, ulcerative colitis, pouchitis)	Modulation of immune response, modulation of gut microbiota
Prevention of heart diseases/influence on blood cholesterol levels	Assimilation of cholesterol by bacterial cells, deconjugation of bile acids by bacterial acid hydrolases, cholesterol-binding to bacterial cell walls, reduction of hepatic cholesterol synthesis and/or redistribution of cholesterol from plasma to liver through influence of the bacterial production of short-chain fatty acids
Prevention of urogenital tract disorders	Production of antimicrobial substances, competition for adhesion sites, competitive exclusion of pathogens
Prevention/alleviation of diarrhoea caused by bacteria/viruses	Modulation of gut microbiota, production of antimicrobial substances, competition for adhesion sites, stimulation of mucus secretion, modulation of immune response
Prevention/treatment of *Helicobacter pylori* infections	Production of antimicrobial substances, stimulation of the mucus secretion, competition for adhesion sites, stimulation of specific and non-specific immune responses
Relief of lactose indigestion	Action of bacterial β-galactosidase(s) on lactose
Shortening of colonic transit time	Influence on peristalsis through bacterial metabolite production

lactose maldigestion, are considered well-established, while others, such as cancer prevention or the effect on blood cholesterol levels, need further scientific backup (Gill & Guarner, 2004). The mechanisms of action may vary from one probiotic strain to another and are, in most cases, probably a combination of activities, thus making the investigation of the responsible mechanisms a very difficult and complex task. In general, three levels of action can be distinguished: probiotics can influence human health by interacting with other microorganisms present on the site of action, by strengthening mucosal barriers, and by affecting the immune system of the host (Marteau & Shanahan, 2003). Again, the strain specificity of each probiotic effect must be stressed; concerning the prevention and treatment of diarrhoea, for example, only indicative evidence of an overall protective effect against travellers’ and antibiotic-associated diarrhoea exists, while the efficacy of *L. rhamnosus* GG in treating rotaviral diarrhoea has extensively been demonstrated

(Gill & Guarner, 2004; Santosa, Farnworth, & Jones, 2006). Further research to support the health claims attributed to probiotics and to unravell the mechanisms behind them is needed.

Safety Considerations

As viable probiotic bacteria have to be consumed in large quantities, over an extended period of time, to exert beneficial effects, the issue of the safety of these microorganisms is of primary concern (Senok et al., 2005). Historical data indicate that lactobacilli and bifidobacteria are safe for human use (Reid, 2005). It has been suggested that the human origin of a strain confirms its normal commensal nature, and, therefore, its safety. However, it remains difficult to establish the origin of a bacterial species, and the fact that infants are born with sterile intestines raises the question whether "human origin" is an appropriate classification for bacteria (Reid, 2005).

Although minor side effects of the use of probiotics have been reported, infections with probiotic bacteria rarely occur and invariably only in immunocompromised patients or those with intestinal bleeding (Gueimonde, Frias, & Ouwehand, 2006; Marteau, 2002; Reid, 2005).

An issue of concern regarding the use of probiotics is the presence of chromosomal, transposon-, or plasmid-located antibiotic resistance genes amongst the probiotic microorganisms. At this moment, insufficient information is available on situations in which these genetic elements could be mobilised, and it is not known if situations could arise where this would become a clinical problem. When dealing with the selection of probiotic strains, the FAO/WHO Consultancy recommends that probiotic microorganisms should not harbour transmissible drug resistance genes encoding resistance to clinically used drugs (FAO/WHO, 2001).

For the assessment of the safety of probiotic microorganisms and products, FAO/WHO has formulated guidelines, recommending that probiotic strains should be evaluated for a number of parameters, including antibiotic susceptibility patterns, toxin production, metabolic and haemolytic activities, and infectivity in immunocompromised animals (FAO/WHO, 2002; Reid, 2005; Senok et al., 2005).

Application of Probiotics in Fermented Meat Products

Fermented Meat as a Carrier for Probiotic Bacteria

Dry fermented meat products are usually not or only mildly heated, which is adequate for the carriage of probiotic bacteria (Ammor & Mayo, 2007; Arihara, 2006). Although there are in principle no major reasons preventing application of probiotic LAB strains in meat, several points have to be carefully addressed.

Although meat is a food with high nutritional value, some consumers may perceive meat products as unhealthy (Arihara, 2006). This can be ascribed to the image of meat as such, in combination with the presence of nitrite, salt, and fat. Meat products are seldomly perceived as "healthy foods", which may compromise their marketing potential (Lücke, 2000). However, adding nutritional assets to meat products could be a strategy to promote them as valuable elements of a high quality diet and to meet the trend for healthier meat products (Arihara, 2006; Jiménez-Colmenero, Carballo, & Cofrades, 2001).

In addition, the impact of the meat environment, with its high content in curing salt and its low water activity and pH, and of meat fermentation technology, based on acidification and drying, on the viability of the cells must be taken into account.

The approaches followed up till now can be summarized as follows: 1) screening for probiotic properties among bacteria that are naturally present in the meat or that originate from meat starter cultures, 2) application of existing probiotic LAB in meat products, 3) evaluating the impact of probiotic sausages on humans during clinical studies, and 4) assessment of the technological suitability of probiotic LAB during sausage-making, in particular with respect to sensory deviations (Leroy, Verluyten, & De Vuyst, 2006).

Screening for Probiotic Properties Among Meat-Associated Bacteria

A promising strategy for the development of probiotic fermented sausages consists of using bacteria that are commonly associated with the meat environment and that possess probiotic properties. In this way, sausage isolates (Klingberg, Axelsson, Naterstad, Elsser, & Budde, 2005; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003; Pennacchia et al., 2004; Pennacchia, Vaughan, & Villani, 2006; Rebucci et al., 2007) or existing commercial meat starter cultures (Erkkilä & Petäjä, 2000) are screened for probiotic properties.

Frequently, the following characteristics are mentioned as indicators for probiotic activity: tolerance to the low pH of gastric juice, resistance to the detergent-like action of bile salts, adhesion to the intestinal mucosa for temporary ileum colonisation, growth capability in the presence of prebiotic carbohydrates, antimicrobial activity towards intestinal pathogens, and nutraceutical properties such as the production of vitamins and conjugated linoleic acid (Ammor & Mayo, 2007; Pennacchia et al., 2006). However, the relevance of at least some of these properties can be questioned and conclusions about true probiotic qualities require caution (see below).

Following this approach, the commercial meat starter strains *L. sakei* Lb3 and *Pediococcus acidilactici* PA-2 have been proposed as potential probiotic starter cultures because of their survival capacities under simulated gastrointestinal conditions (Erkkilä & Petäjä, 2000). Also, isolates of *L. casei/paracasei* from sausages fermented with *L. casei*, *L. paracasei*, *L. rhamnosus*, and *L. sakei* were screened for viability in artificial gastric juice, artificial intestinal fluid, in vitro adhesion to human intestinal cell lines, organic acid production, and pathogen inactivation (Rebucci et al., 2007). Several *L. plantarum* sausage isolates were found to have appreciable adhesion rates towards Caco-2 cell lines and were considered as better adhesive bacteria than *L. brevis* and *L. paracasei*–group sausage isolates (Pennacchia et al., 2006).

It is important to note that the obtained results of the latter studies are preliminary and that further research is needed to prove the true probiotic health nature of the candidate strains obtained.

Use/Application of Known Probiotic Strains

As an alternative to the approach mentioned above, it may be investigated if strains with (presumed) probiotic properties perform well in a fermented meat environment. Such strains are usually human intestinal isolates and hence not from meat origin. Therefore, they should be able to compete with the natural meat microbiota in an environment which is not their natural habitat, be able to survive the fermentation and drying process, and, preferably, be able to grow to numbers that display health-promoting effects. Alternatively, micro-encapsulation in alginate beads may be used to increase survival (Muthukumarasamy, & Holley, 2006, 2007). In this way, several lactobacilli of human intestinal origin have been shown to survive the sausage manufacturing process and can be detected in high numbers in the end-product (Arihara et al. 1998; Erkkilä, Petäjä, et al., 2001; Erkkilä, Suihko, Eerola, Petäjä, & Mattila-Sandholm, 2001; Pidcock, Heard, & Henriksson, 2002; Sameshima et al., 1998).

It is certainly an asset if these new meat starter cultures also contribute to food safety. Probiotic strains, with additional food safety assets, could contribute a high added value to healthy fermented meat products. For instance, *L. reuteri* ATCC 55730 and *Bifidobacterium longum* ATCC 15708 increased the inactivation of *Escherichia coli* O157:H7 during sausage manufacturing (Muthukumarasamy & Holley, 2007). *L. rhamnosus* FERM P-15120 and *L. paracasei* subsp. *paracasei* FERM P-15121 inhibited the growth and enterotoxin production of *Staphylococcus aureus* to the same extent as a commercial *L. sakei* starter culture (Sameshima et al., 1998). On the other hand, *L. acidophilus* FERM P-15119 could not satisfactorily decrease *Staph. aureus* numbers, indicating the importance of careful strain selection with respect to both probiotic and food safety properties.

Human Studies

Ultimately, human studies should confirm the functionality of probiotic fermented sausages. In contrast to the dairy industry, such studies are very scarce till date and results have been moderately successful. One study deals with the effect of probiotic sausages on immunity and blood serum lipids. The daily consumption of 50 g of probiotic sausage by healthy volunteers, containing *L. paracasei* LTH 2579, during several weeks has been shown to modulate various aspects of host immunity but there was no significant influence on the serum concentration of different cholesterol fractions and triacylglycerides (Jahreis et al., 2002). In faecal samples, there was a statistically significant increase in the numbers of *L. paracasei* LTH 2579, but not in the faeces of all volunteers. It is interesting to mention that the sausage matrix seems to protect the survival of probiotic lactobacilli through the gastrointestinal tract (Klingberg & Budde, 2006).

Technological Suitability

In all cases, it should be checked that the sensory properties of the end-products are not negatively affected, especially when strains from non-meat origin are used. The (potential) probiotic strains *L. rhamnosus* GG, *L. rhamnosus* LC-705, *L. rhamnosus* E-97800 and *L. plantarum* E-98098 have been tested as functional starter culture strains in Northern European sausage fermentation without negatively affecting the technological or sensory properties, with a (minor) exception for *L. rhamnosus* LC-705 (Erkkilä, Petäjä, et al., 2001; Erkkilä, Suihko, et al., 2001). Similarly, the intestinal isolates *L. paracasei* L26 and *B. lactis* B94 had no negative impact on the sensory properties of the product when applied in conjunction with a traditional meat starter culture (Pidcock et al., 2002). Also, the use of alginate-microencapsulation of *L. reuteri* was not resulting in differences concerning sensory quality (Muthukumarasamy, & Holley, 2006).

Conclusions and Critical Remarks

Although meat products containing probiotic LAB are already being marketed since 1998 by German and Japanese producers (Arihara, 2006), most scientific results obtained until now are rather preliminary and mostly based on incomplete approaches, not permitting full assessment of the probiotic effects of fermented sausages on human health.

In vitro studies are valuable tools to asses the survival of potential probiotic strains in the human gastrointestinal tract. Furthermore, they can provide insight into the abilities of a strain to adhere to surfaces and to inhibit growth or adhesion of pathogens. However, the mere ability of a strain to survive the passage through the human intestinal tract does not qualify a microorganism as a probiotic. Inhibition studies might help to elucidate the mechanisms behind a probiotic effect, but in vitro inhibition of pathogens by a potential probiotic by no means guarantees that the same will occur in the complexity of the colon ecosystem. When only based on results of in vitro studies, the use of the term "potential" probiotic is questionable. Research regarding the launch of new probiotic strains should rigorously follow the guidelines formulated by the FAO/WHO (2002), including detailed identification of the strain and an approved beneficial health effect.

Nevertheless, the addition of microorganisms with known probiotic characteristics to a meat fermentation process seems an elegant solution for the development of probiotic meat products. Most research concerning this strategy focuses on the survival of the added species in the meat matrix and its influence on the technological and sensory characteristics of the final product. However, the influence of the carrier (meat matrix) and its interactions with the microbial cells on the beneficial effects exerted by a probiotic strain must be assessed. It is recommended that the functionality of each probiotic strain is documented independently in each final formulation (Mercenier et al., 2003).

Finally, one must not overlook that meat, in particular, cured meat, might not be the most obvious carrier for probiotic microorganisms, as compared to dairy products, because of its negative connotations and potential health implications in the Western diet.

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