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# Probiotic fermented sausages: Myth or reality?

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#### Abstract

Experts view the development of functional foods as an opportunity for innovation for the meat industry in line with consumers' concerns on food nutrition, well being and food safety. The main microbiota of fermented sausages consists of lactobacilli, which suggests that properly characterized strains could be selected as starter cultures. The selection of strains from human origin was considered in order to promote their survival in the host. *Lactobacillus rhamnosus* CTC1679 dominated during the ripening process and temporarily colonized the gastrointestinal tract of healthy volunteers, confirming that this strain could be delivered as a potential probiotic in fermented sausages.

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#### 1. Introduction

Meat fermentation is an ancient preservation method resulting in microbiologically safe products with particular sensory attributes and unique and distinctive meat properties. Fermented meats play an important role in many diets and are very appreciated by the consumer. They are a source of protein, fat, essential amino acids, minerals, vitamins and other functional nutrients. Currently, consumers are very conscious about their nutrition and well being, but regrettably often associate processed meat products as being high fat, high sodium chloride and cancerpromoting food and, as a consequence, ingestion of meat and meat products is avoided or limited. As an opportunity to improve this perception, in recent years, researchers and the meat processing sector have put much effort in

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developing and marketing functional meat products, i.e., foods used to prevent and treat certain disorders and diseases, in addition to their nutritional value per se<sup>1</sup>.

An attractive strategy for designing functional meat products is the use of probiotic cultures. Probiotic bacteria are 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'<sup>2</sup>. Those beneficial health effects can be multiple and depend on strain, dose, administration method, population group and physiological state of the host<sup>3</sup>. Dairy products are the most commonly used food vehicles for the delivery of probiotics. They have been on the market for many years and are well accepted by consumers<sup>4</sup>. However, a few studies have also demonstrated the feasibility of elaborating fermented meat products with probiotic cultures (e.g.<sup>5,6,7</sup>).

Most of the microorganisms used for probiotic purposes belong to the *Lactobacillus* and *Bifidobacterium* genus although the probiotic nature of other QPS (with Qualified Presumption of Safety) species (e.g. *Lactococcus*, *Enterococcus*, *Sacharomyces*, *Propionibacterium*, *Bacillus*, etc.) has also been reported<sup>8</sup>. Lactic acid bacteria (LAB) are currently used for the production of a great variety of fermented foods derived from plant and animal products and are also usual inhabitants (true commensals or transient passengers) of the gastrointestinal (GI) tract<sup>9</sup>. Characteristics of probiotic bacteria include resistance to the acidic conditions of the stomach and to bile acids and pancreatin from the beginning of the small intestine. Other key traits are the production of antimicrobial compounds (e.g. bacteriocins), the adhesion to the intestinal mucosa, the non production of biogenic amines and the absence of specific antibiotic resistances<sup>2</sup>.

The work presented here summarises the research of our group on starter cultures for fermented meat products with the final aim of obtaining functional fermented sausages with a satisfactory overall sensory quality and safety as a potential way for delivering probiotic bacteria.

#### 2. Isolation and characterization of LAB from intestinal origin

From a total of 109 LAB isolated from infant faeces (<6 months of age) we observed that *Lactobacillus* was the most prevalent genus (48% of the isolates) followed by *Enterococcus*, *Streptococcus* and *Weissella*. At the species level, *Lactobacillus gasseri* (21%), *Lactobacillus casei/paracasei* (10%) and *Enterococcus faecalis* (38%) were among the most predominant 10. As *Lactobacillus* is the most technologically relevant genus in fermented sausages, further selection was focused on it. RAPD-PCR allowed the discrimination of identical strains which were further evaluated for their ability to grow *in vitro* in the processing conditions of fermented sausages (presence of NaCl, nitrite and acidity) and for their functional (antagonistic activity against *Salmonella* and *L. monocytogenes*, resistance to the GI tract conditions and aggregation capacity) and safety properties (antibiotic susceptibility and tyramine production). Considering all the results, *L. casei/paracasei* CTC1677 and CTC1678, *Lactobacillus rhamnosus* CTC1679, *L. gasseri* CTC1700 and CTC1704 and *Lactobacillus fermentum* CTC1693 were selected as potential probiotic strains and their ability to lead the fermentation (9 days at 15°C) in model sausages was tested. Despite LAB counts being similar in all batches, not all the assayed strains were competitive in that environment and only *L. casei/paracasei* and *L. rhamnosus* strains achieved 100% implantation and acidified the mixture. Thus, *L. casei/paracasei* CTC1677, *L. casei/paracasei* CTC1678 and *L. rhamnosus* CTC1679 were able to lead the fermentation and become dominant over the endogenous LAB, confirming their suitability as starter cultures.

#### 3. Designing safe probiotic fermented sausages

When the suitability of the previously selected strains (CTC1677, CTC1678 and CTC1679) as potential probiotic cultures was assayed in nutritionally enhanced (with reduced sodium and fat content) low-acid fermented sausages (fuets), we observed that only L. rhamnosus CTC1679 was able to grow and dominate (levels ca. 10<sup>8</sup> cfu/g) the endogenous LAB during ripening in two independent trials (Fig. 1), thus being more competitive than the commercial probiotic strains L. plantarum 299v, L. rhamnosus GG and L. casei Shirota<sup>11</sup>. At the sensory level, no significant differences were observed among fuet type sausages elaborated with the different starter cultures regarding most of the evaluated sensory attributes. All the products recorded a satisfactory overall sensory quality without any noticeable off-flavour, and with the characteristic sensory properties of low-acid fermented sausages.

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