
Features and functional properties of lactic acid bacteria used as biological preservatives of meat processing: A review article

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Due to consumers' preference for natural preservatives in foods, there have been intensified efforts to shift from the usual chemical preservatives. Focus of food processors has now been on the use of biological agents in the preservation of foods. This development has, however, necessitated intense research activities towards the realisation of this objective. Processing of fermented meat products is based on the use of starter cultures, notably lactic acid bacteria, which assist in initiating rapid acidification by their ability to produce organic acids. Strains of lactic acid bacteria (LAB) with industrial applications as starter cultures have been available performed while others are being developed. LAB can contribute to safety of foods by competitive exclusion of spoilage and pathogenic organisms due to their antimicrobial activity. They also offer some organoleptic, technological, nutritional or health advantages. The current review reports on features and functional properties of lactic acid bacteria used as biological preservatives of meat products.

Key words: natural preservatives, biological agent, chemical preservatives, lactic acid bacteria, antimicrobial activity

Introduction

Meat is very rich in all food classes and as such is prone to spoilage by microbial activities. Some of the ways to preserve it is to process into various forms such as sausages for consumption. The sausages are usually subjected to fermentation by starter cultures in an effort to prolong the shelf life. Fermented sausage is a mixture of comminuted fat and meat, salt, nitrate and/or nitrite, sugar and spices, which is stuffed into casings, subjected to maturation and then allowed to dry (Hugas and Monfort, 1997). The attribute of the final product is largely dictated by the ripening process during maturation and the process confers on the product its particular ease of slicing, firmness, colour

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and flavour. The maturation process is characterized by complex interactions of chemical and physical reactions associated with the microbiological development of the typical ecological microflora (Ordóñez *et al.*, 1999). Biopreservation of meat through spontaneous fermentation involves the participation of lactic acid bacteria (LAB), non-coagulase producing Cocci (NPC) especially species of *Staphylococcus* and yeasts and moulds. Commercial meat starter cultures contain mixtures of LAB and NPC. Meat starter cultures are preparations that contain active or dormant microorganisms that develop the desired metabolic activity in the meat. They are responsible for the change associated with organoleptic properties of many food products especially meats (Hammes *et al.*, 1990). The LAB of meat origin are particularly well adapted to the ecological niche of meat fermentation and thus should be considered for selection as starter cultures. Phenotypic methods relying on physiological or biochemical criteria have been widely used for LAB identification and to overcome the laboriousness involved in the methods, molecular techniques such as rRNA hybridization probes, species-specific PCR, PCR-denaturing gel electrophoresis, real-time PCR have been developed for LAB species identification (Furet *et al.*, 2004; Aymerich *et al.*, 2006). Randomly amplified polymorphic DNA (RAPD)-PCR analysis has been used to estimate the biodiversity among LAB (Aymerich *et al.*, 2006).

Some LAB genera that have been identified from meat products include *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Weissella* and *Enterococcus* (Martín *et al.*, 2005; Aymerich *et al.*, 2006), with the first genera being dominant. *L. sakei*, *L. curvatus*, *L. plantarum*, *Lactobacillus pentosus*, *Lactobacillus casei*, *Pediococcus pentosaceus* and *P. acidilactici* are the species most used as commercial meat LAB starter cultures (Hammes and Hertel, 1998). In designing a starter culture for a meat bioprocessing, there is need to first isolate and characterize the LAB strains from the intended meat product and then selection of suitable strains follows. For example the main function of LAB is to obtain a rapid pH drop of the batter, which in turn favours product safety by inactivating pathogens; product stability and shelf life by inhibiting undesirable changes caused by spoilage microorganisms; and creates the biochemical conditions to attain the new sensory properties of the ripe products through modification of the raw materials. The use of starters as functional flora is presently of immense importance worldwide as they help in optimizing meat fermentation processes and to produce products of good organoleptic and probiotic qualities. The objective of the current review was to report various features and functional properties of LAB used in the biopreservation of meat products.

Features of LAB as biological preservatives

LAB are expected to produce of lactic acid from carbohydrate sources rapidly in adequate concentrations. This is the main role LAB in meat bioprocessing and depends on several chemical, physical and microbiological reactions. During acidification of the meat products, LAB bring about the coagulation of muscle proteins, resulting in the increased slice stability, firmness and cohesiveness of the final product (Ordóñez *et al.*, 1999). They also enhance the spontaneous reduction of nitrites to nitric oxide, which reacts with the myoglobin to form nitrosomyoglobin, the compound responsible for the typical pink colour of meat products (Hugas and Monfort, 1997). Furthermore, LAB contribute to the flavour of the final product through the formation of noticeable acidic and vinegary tastes. Production of organic acids is the determining factor on which the shelf life and the safety of the final product depend (Olaoye *et al.*, 2008; Olaoye and Onilude, 2009). The inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids. It has also been observed that a rapid decrease in pH caused by amine-negative starter cultures can largely prevent biogenic amine (BA) accumulation in sausages (Maijala *et al.*, 1993). The immediate and rapid formation of acid at the beginning of the fermentation process, and the production of sufficient amounts of organic acids allowing a pH below 5.1 to be reached, are therefore essential requirements of meat LAB starters (Olaoye *et al.*, 2008). Excessive acid formation, however, is often associated with colour defects (due to the inhibition of the NPC) and sometimes with gas formation – one of the most important problems in sausage fermentation (Buckenhüskes, 1993). The ability of the LAB culture to compete with the natural microbiota of the raw material and to undertake the metabolic activities expected is conditioned by its growth rate and survival in the conditions prevailing in the meat product. Such conditions are an anaerobic atmosphere, moderate salt concentrations, low temperatures and low pH. The salt concentration of about 2% in the meat product can reach 15% in the final product. In sausage production, the manufacturing temperature ranges from 4 to 7 °C when preparing the batter, from 18 to 24 °C during the fermentation period and from 12 to 15 °C during the drying and ripening period (Montel, 1999). The initial pH of the batter, which is generally around 6.0 decreases during fermentation and reaches values about 4.8. The yeasts increase the pH of the product (Cook, 1995), achieving final values of 5.2. Hence the growth rate at different temperatures, the tolerance of salt concentrations of 2–10% and of pHs in the range 4.5–6.0 are limiting factors affecting the persistence and competitiveness of the starter culture over the entire fermentation and ripening process. *L. sakei* can grow at 4 °C, in the presence of 6.5% NaCl, and

at pH 4.2 (Ammor *et al.*, 2005). At 15 °C and in the presence of about 2% NaCl, the meat-borne LAB strain shows growth rates which allow 0.55 generations to be produced in one hour. Its psychrotrophic character and salt tolerance may be due to its ability to efficiently accumulate osmo- and cryoprotective solutes such as betaine and carnitine, and to its cold stress response: *L. sakei* has more putative cold-stress genes than any other lactobacilli (Chaillou *et al.*, 2005). A combination of mechanisms, including modification of carbohydrate metabolism (down-regulation of glycolysis) and stimulation of oxidative stress may also increase its resilience to cold.

Heterofermentative LAB have been noted to be unsuitable for meat fermentation because of the associated formation of large amounts of carbon dioxide which leads to holes of different sizes in the product (Buckenhüskes, 1993). Also these LAB produce concentrations of acetic acid that cause a pungent off flavour. Most lactobacilli are able to form hydrogen peroxide by oxidizing lactate. Hydrogen peroxide can interfere with the organoleptic properties of fermented meat products by increasing rancidity as well as discoloration of the final product. Catalase is an enzyme that hydrolyses hydrogen peroxide; some LAB strains involved in meat fermentations such as *L. sakei*, *L. plantarum*, *L. pentosus* and *P. acidilactici*, possess heme-dependent catalase activity which is active in meat products since these substrates contain haemin in abundance (Ammor *et al.*, 2005). This property is a very desirable feature of meat LAB starter cultures. It has been noted earlier that while decreasing the pH of the meat matrix, LAB participate in the formation of the typical pink colour through the spontaneous reduction of nitrites to nitric oxide. Some meat LAB strains have also been reported to possess nitrate reductases and heme-dependent and heme-independent nitrite reductases which are directly involved in the mechanisms of nitrosomyoglobin formation. LAB have only weak proteolytic action on myofibrillar proteins (Sanz *et al.*, 1999a), however, some species of *Lactobacillus* (e.g. *L. plantarum* and *L. sakei*) strains actively contribute to the hydrolysis of the sarcoplasmic proteins (Sanz *et al.*, 1999b) and to the subsequent decomposition of peptides into amino acids. Several peptidase activities have been reported in LAB strains isolated from fermented meat products (Fadda *et al.*, 1999). Also some *L. sakei*, *L. curvatus* and *L. plantarum* strains possess leucine and valine amino-peptidases, which contribute to the catabolism of proteins and peptides generating free amino acids which are precursors of flavour compounds in the final meat product (Papamanoli *et al.*, 2003). Evaluation of proposed LAB starter cultures to be used on meat processes for proteinases, peptidases and amino-peptidases activities is very vital to the selection of final starters.

It has been noted that meat starter cultures are mainly mixtures of LAB and NPC and hence in order to perform their expected functions, LAB starters must be able to show synergy with NPC starter components. In addition to inhibition of unwanted flora, some LAB starters have been observed to antagonise other organism used as starters such as *Kocuria varians* (Hammes *et al.*, 1990). It becomes imperative that microbial strains intended for use in any starter mixture should be assessed for antagonism against co-starters.

Biopreservation and safety issues

One of the major functions of starter cultures is to improve safety usually mediated by antagonism against pathogenic and spoilage microorganisms through production of organic acid and bacteriocins. It is also important that starter cultures show no tendencies whatsoever of being pathogenic or toxinogenic (Hammes and Hertel, 1998). In addition there is need to prevent antibiotic resistance and the production of biogenic amines by the proposed starters. LAB starter cultures have a primary role of rapid production of organics acids which inhibits the growth of unwanted flora and enhances product safety and shelf-life. The antimicrobial effect of organic acids lies in the reduction of pH and in the action of undissociated acid molecules (Podolak *et al.*, 1996). It has been proposed that low external pH causes acidification of the cytoplasm while the lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane causing a collapse in the electrochemical proton gradient. In the alternative cell membrane permeability may be affected leading to disruption of substrate transport systems (Snijders *et al.*, 1985). The concentrations and types of organic acids produced during the fermentation process depend on the LAB strains present, the culture composition, and the growth conditions (Lindgren and Dobrogosz, 1990). The L(+) lactic acid is more inhibitory than its D(+) and so only strains producing mainly L(+) type need be selected as starters (Buckenhüskes, 1993). Besides, the D(+) isomer is not hydrolyzed by human lactate dehydrogenase and may cause health problems.

Interest in the bacteriocins produced by meat LAB increased dramatically in recent times indicating the increase in their importance with respect to the functional properties. Many bacteriocins are produced by some LAB species involved in meat fermentation processes (Tichaczek *et al.*, 1992; Enan *et al.*, 1996). Meat borne LAB produce a range of bacteriocins that are generally active towards other LAB, especially closely related strains, and food borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Bacillus cereus* (Messi *et al.*, 2001; Noonpakdee *et al.*, 2003). Bacteriocins exert their inhibitory action via formation of pores in the cytoplasmic membrane of cells of sensitive organisms. Gram-negative

bacteria are protected by their outer membrane, which prevents bacteriocins from reaching the plasma membrane (Abee *et al.*, 1995). It is generally accepted that bacteriocin activity is less effective in fermented meats than in *in vitro* systems. The less effectiveness may be caused by the binding of the bacteriocin molecules to food components (especially fat matrix), and by the destabilizing action of proteases and other enzymes. Other limitations of bacteriocin effectiveness include uneven distribution in the food matrix and their inhibition by salt and curing agents (Leroy and de Vuyst, 1999). Notwithstanding, there have been interesting reports of many bacteriocin producing meat LAB being used as bioprotective cultures to prevent the growth of pathogens in sausage. For example, the use of bacteriocin-producing *L. sakei* as a starter culture decreases the numbers of *Listeria* in fermented meat (Hugas *et al.*, 1995; Hugas *et al.*, 1996). Antilisterial effects have also been demonstrated with bacteriocinogenic *L. curvatus* (Hugas *et al.*, 1996), *L. plantarum* (Campanini *et al.*, 1993; Dicks *et al.*, 2004) and *P. acidilactici* (Luchansky *et al.*, 1992). The production of bacteriocins with a broad inhibition range, especially towards food-borne pathogens is therefore highly desirable as this would ensure the competitiveness of the starter strain while reducing the numbers of associated harmful and unwanted microorganisms. Limiting the transmission of antibiotic resistance genes to unrelated pathogenic or opportunistic bacteria is essential because resistance to antibiotics has presented a growing worldwide public health problem. The food chain has been recognized as one of the main routes for the transmission of antibiotic resistant bacteria between animal and human populations. Authorities in many developed countries have recently concluded that some bacteria used for or in feed production might pose a risk to human and animal health because of harbouring strains with transferable resistance genes (European Commission, 2005). Fermented meats that are not heat-treated before consumption provide a vehicle for such bacteria and can act as a direct link between the indigenous microflora of animals and the human gastro intestinal tract (GIT).

In some studies, food-associated bacteria such as *L. sakei*, *L. curvatus*, *Leuconostoc mesenteroides*, and *P. pentosaceus* have been isolated from human faeces, suggesting their ability to survive passage through the human GIT (Walter *et al.*, 2001). Also studies have reported antibiotic resistance in LAB from meats and meat products; a few strains involved in sausage fermentation such as *L. curvatus* and *L. plantarum* have been found to show varying levels of resistance (Teuber and Perreten, 2000; Gevers *et al.*, 2003). Although most of these resistances have been characterized as intrinsic, some genetic determinants such as chloramphenicol acetyltransferase, *cat-TC*, erythromycin, *erm(B)*, and tetracycline, *tet(M)*, *tet(S)*, resistance genes have

been identified, suggesting the possibility of occurrence of horizontal gene transference (Lin *et al.*, 1996; Gevers *et al.*, 2003). Another study showed some strains of lactobacilli harbouring transferable resistance genes due to their resistance levels to chloramphenicol, erythromycin, tetracycline and oxacillin (Danielsen and Wind, 2003). It is therefore necessary that bacterial strains being used in biopreservation processes be verified as not harbouring resistance genes. Biogenic amines (BA) are organic bases with aliphatic, aromatic or heterocyclic structures, found in several foods, and are mainly produced by the microbial decarboxylation of amino acids. If consumption of these amines is excessive, it could cause nervous, gastric, intestinal, and blood pressure problems (Suzzi and Gardini, 2003). Nowadays, increasing attention is given to BA because of sensitive consumers and in such people the action of amine oxidases, the enzymes involved in the detoxification of these substances, is normally deficient. Accumulation of BA in foods requires the presence of precursors (amino acids), microorganisms with amino acid decarboxylase activity, and favourable conditions for growth and decarboxylation. The large quantities of protein present and the proteolytic activity seen during meat ripening provide the precursors for later decarboxylase reactions performed by both starter cultures and wild microbiota (Suzzi and Gardini, 2003). Most strains of *L. curvatus*, one of the main species used as a starter in sausage production, are associated with high BA production (Pereira *et al.*, 2001). An important requirement in the selection of LAB starter cultures for fermented meat production is that they show no amino decarboxylase activity. Rapid growth and acid production will further prevent the development of wild amine-producing microflora. Some authors have reported on the ability of selected *L. sakei* to greatly reduce BA accumulation in fermented sausages (Bover-Cid *et al.*, 2001; González-Fernández *et al.*, 2003). The introduction of starters with amine oxidase activity might be a means to reducing the amount of biogenic amines produced *in situ*, as noted in some LAB cultures involved in meat fermentations (Fadda *et al.*, 2001).

LAB cultures as probiotics

LAB cultures could function as probiotics which are non-pathogenic microorganisms that when ingested in certain numbers exert a positive influence on host physiology and health beyond inherent general nutrition. A large quantity of these bacteria are consumed i) to ensure a healthy microbial balance in the intestine ii) to increase the returns of their beneficial activities and iii) to counteract the action of harmful populations (Ouwehand *et al.*, 2002). Since meat products are not usually heated, they are thought to be adequate for the carriage of probiotics (Incze, 1998). More over, probiotic meat

starter cultures which do not alter the technological and sensory properties of the products have been proposed and used in the manufacturing of fermented meat products (Erkkilä *et al.*, 2001). Some of the important properties of probiotic cultures include acid and bile salt resistance, without which the probiotic microorganisms could not reach the human intestine, where they are expected to exert their health promoting effects. The ability of the strains in attaching to the intestinal mucosa is very important for promoting changes in intestinal ecology (Erkkilä and Petaja, 2000). The ability of bacteria to survive in the gastric juice depends on their capability to tolerate low pH. The transit time can be from 30 min to 4 h depending on the individual, the diet and other reigning conditions. A number of LAB isolated from fermented meat sources such as *Pediococcus acidilactici* and *P. pentosaceus*, can tolerate such acidic conditions (Klingberg *et al.*, 2006). It may thus be imperative that strains intended for probiotic purposes should be screened for pH tolerance in acidified culture media for required length of time. It needs be stressed that most bacteria that survive the acidic conditions in the stomach must then combat with the detergent-like function of the bile salts released into the duodenum after the ingestion of fatty meals. Most microorganisms have the ability to reduce the emulsifying effect of the bile salts by some processes, thus decreasing their solubility (Erkkilä and Petaja, 2000). Also some LAB strains have been shown to resist bile salts. The cells of proposed probiotic bacteria need to adhere to the mucosa and colonise the ileum, where probiotics are believed to exert their beneficial effects (Ouwehand *et al.*, 2002). The ability of potential probiotic meat LAB strains to colonise the human GIT has been studied *in vitro* (Klingberg *et al.*, 2006). It is thus important that probiotic starter strains be screened for adherence and persistence in the human GIT. The antimicrobial activity displayed by potentially probiotic meat LAB strains towards pathogenic microorganisms has to be functional under anaerobic conditions. Some *Lactobacillus* strains can inhibit the growth of *L. monocytogenes* strains as well as enterohaemorrhagic *Escherichia coli*, and strains of *Salmonella* Typhimurium, *B. cereus* and *Shigella sp* (Klingberg *et al.*, 2006). Lactic acid bacteria are noted to be ideal cellular factories for the production of nutraceutical compounds (Hugenholtz and Smid, 2002). Development in the genetic recombinant of LAB underscores the possibility of developing meat LAB starter cultures for the *in situ* production of vitamins via the over-expression or disruption of relevant metabolic genes (Sybesma *et al.*, 2003; Sybesma *et al.*, 2004). Many other species involved in meat fermentation have been engineered to produce excess of vitamin B11 (Sybesma *et al.*, 2003). This is believed to reduce help maintain normal plasma homocysteine levels and cognitive functions, as well as to provide protection against certain forms

of cancer. The technique could permit the possibility of fortifying meat products with vitamins and other essential compounds and thereby producing healthier meat products.

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