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Review Paper

Spoilage and preservation of meat: a general appraisal and potential of lactic acid bacteria as biological preservatives

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Spoilage of meat has remained a serious challenge in developing countries, including Nigeria, for decades. This has been due to poor storage systems in such countries where necessary facilities that could help promote preservation are unavailable. Where available, unsteady power supply necessary to maintain such facilities has constituted a serious problem, thereby rendering them to function below their maximum capacity. Furthermore, the ambient temperature in developing countries that are in tropical regions is usually about 30°C or above; most spoilage organisms have been found to have their optimum growth temperature within such temperature range. In the present review, a general appraisal of meat spoilage and the potential of lactic acid bacteria in its biopreservation are discussed, with the view to suggesting a way to reduce wastage normally associated with meat due to spoilage. This could be of tremendous importance in developing countries, such as Nigeria, where procurement and maintenance of storage facilities have remained a matter of serious concern for many meat processors till date.

Keywords: challenge, storage systems, unsteady power supply, spoilage organisms, biopreservation, meat processors

INTRODUCTION

Meat is a nutritious, protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used (Olaoye and Onilude, 2010). Shelf life and maintenance of the meat quality are influenced by a number of interrelated factors including holding temperature, which can result in detrimental changes in the quality attributes of meat. Spoilage by microbial growth is the most important factor in relation to the keeping quality of meat (Lambert *et al*. 1991).

In most developing countries, including Nigeria, fresh meat forms a significant proportion of meat intake (Olaoye and Onilude, 2010). It is either eaten cooked or processed into other forms to avoid associated spoilage. The main causative factor of such spoilage has been linked to unavailability of necessary storage facilities and favourable ambient temperature that usually prevail in developing countries that are in tropical regions (Olaoye *et al*., 2010). Research findings have suggested that there is increasing attention on the use of naturally occurring metabolites produced by selected lactic acid bacteria (LAB) to inhibit the growth of spoilage microorganisms (Onilude *et al*. 2002; Olaoye and Onilude, 2010; Olaoye *et al*., 2010; Olaoye and Dodd, 2010). These authors have demonstrated the potential of LAB cultures as biopreservatives during processing and preservation of many forms of meat products. Lactic acid bacteria growing naturally in foods produce antimicrobial substances such as lactic and acetic acids, diacetyl, hydrogen peroxide and bacteriocins (Olaoye *et al.* 2008).

A general appraisal on the spoilage of meat and possible preservation by the application of LAB as biological preservatives are presented in this review.

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Spoilage and preservation of meats

Factors affecting meat spoilage

Meat has long been considered a highly desirable and nutritious food, but unfortunately it is also highly perishable because it provides the nutrients needed to support the growth of many types of microorganisms (Kolalou *et al*., 2004). Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption. In general, the metabolic activity of the ephemeral microbial association which prevails in a meat ecosystem under certain aerobic conditions, or generally introduced during processing, leads to the manifestation of changes or spoilage of meat (Nychas *et al*., 2008). These changes or spoilage are related to the (i) type, composition and population of the microbial association and, (ii) the type and the availability of energy substrates in meat. Indeed the type and the extent of spoilage is governed by the availability of low-molecular weight compounds (e.g., glucose, lactate) existing in meat (Nychas *et al*., 1998; Nychas and Skandamis, 2005). By the end of the phase changes and subsequently, overt spoilage is due to catabolism of nitrogenous compounds and amino acids as well as secondary metabolic reactions

The post-mortem glycolysis, caused by indigenous enzymes, ceases after the death of the animal when the ultimate pH reaches a value of 5.4–5.5 (Olaoye, 2010). Afterwards, the contribution of meat indigenous enzymes in its spoilage is negligible compared to the microbial action of the microbial flora (Tsigarida and Nychas, 2001). A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen (O_2), indigenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms. All of these factors, either alone or in combination, can result in detrimental changes in the colour, odour, texture and flavour of meat. Fresh meat has a shelf life of 1 day or less at ambient storage temperatures, 20-30°C (Lambert *et al*., 1991).

Spoilage is said to be a state of a particular food in which it is offensive to consumers' senses, usually caused by metabolites of contaminant microorganisms (Paulsen and Smulders, 2003). Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odours, and the development of slime would constitute the main qualitative criteria for meat rejection. In general, spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual and the intensity of the change (Nychas *et al*., 2008). Spoilage of meat can be considered as an ecological phenomenon that encompasses the changes of the available substrata, such as low molecular weight compounds, during the proliferation of bacteria that constitute the microbial association of the stored meat (Nychas *et al*., 2007). The prevailing of a particular microbial community of meat depends on the factors that persist during processing, transportation and storage in the market. Such may vary widely from one country to another as a result of differences in climatic conditions, coupled with possible varying levels in knowledge of food hygiene practices of the handlers.

The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution. In fact, some of the microorganisms originate from the animal's intestinal tract as well as from the environment with which the animal had contact at some time before or during slaughter (Koutsoumanis and Sofos, 2004). Other organisms, including psychrotrophic bacteria, are recovered from hides and work surfaces within an abattoir as well as from carcasses and butchered meat at all stages of processing (Gill, 2005).

A wide range of micro-organisms coming from different sources are introduced onto carcass surfaces, which contain abundant nutrients and which have high water availability. Only a few of the contaminants will be able to initiate growth, and only some of these will eventually spoil the meat by means of their biochemical attributes. Predominance of different groups of microorganisms on meat depends on the characteristics of the meat, the environment in which meat is stored as well as the processing that meat may undergo (Gill and Molin, 1991). As earlier noted, a vast number of studies in meat microbiology have established that spoilage is caused by only a fraction of the initial microbial association that comes to dominate (Nychas *et al*., 2007). The range of microbial taxa found on meat is given in Table 1. A consortium of bacteria, commonly dominated by *Pseudomonas* spp., is in most cases responsible for spoilage of meat stored aerobically at different temperatures (-1 to 25°C); the *Pseudomonas* spp. can grow under refrigeration temperatures (Stanbridge and Davis, 1998; Koutsoumanis *et al*., 2006). It is established that under aerobic storage three species of *Pseudomonas*, *Ps. fragi*, *Ps. fluorescens* and *Ps. Lundensis*, are the most important spoilage organisms. The population of pseudomonads to the level of 10^{7-8} CFU/g, has been attributed to slime and off-odours formation (Table 2 and 3). However, in practice both these characteristics become evident when the pseudomonads have exhausted the glucose and lactate present in meat and begin to metabolise nitrogenous compounds such as amino acids. This is significant in dry firm dark meat (produced due to exercise preslaughter; Olaoye, 2010) where there is no carbohydrate and therefore spoilage occurs earlier at lower populations (10^6). *Brochothrix thermosphacta* and lactic acid bacteria (LAB) have been detected in the aerobic spoilage flora of

Table 1 Genera of spoilage bacteria commonly found on meats and poultry

Microorganisms	Gram reaction	Fresh	Processed
Achromobacter	-	X	
Acinetobacter	-	XX	X
Aeromonas	-	XX	X
Alcaligenes	-	X	
Bacillus	+	X	X
Brochothrix	+	X	X
Campylobacter	-	X	
Carnobacterium	+	X	
Chromobacterium	-	X	
Citrobacter	-	X	
Clostridium	+	X	
Corynebactenum	+	X	X
Enterobacter	-	X	X
Enterococcus	+	XX	X
Escherichia	-	X	
Flavobacterium	-	X	
Hafnia	-	X	X
Janthinobacterium	-		X
Klebsiella	-	X	
Lactobacillus	+	X	XX
Lactococcus	+	X	
Leuconostoc	+	X	X
Listeria	+	X	X
Microbacterium	+	X	X
Micrococcus	+	X	X
Moraxella	-	XX	
Proteus	-	X	
Providencia	-	X	X
Pseudomonas	-	XX	X
Shewanella	-	X	X
Staphylococcus	+	X	X
Streptococcus	+	X	X
Weissella	+	X	X
Yersinia	-	X	

Source: Nychas *et al.* (2007)

X, known to occur; XX, most frequently isolated

Table 2 Common defects in meat products and causal bacteria

Defect	Meat product	Bacteria
Slime	Meats	*Pseudomonas*, *Lactobacillus*, *Enterococcus*, *Weissella*, *Brochothrix*
H_2O_2 greening	Meats	*Weisella*, *Leuconostoc*, *Enterococcus*, *Lactobacillus*
H_2S greening	Vacuum packaged meat	*Shewanella*
H_2S production	Cured meats	*Vibrio*, Enterobacteriaceae
Sulfide odour	Vacuum Packaged meat	*Clostridium*, *Hafnia*
Cabbage odour	Bacon	*Providencia*
Putrefaction	Ham	Enterobacteriaceae, *Proteus*
Bone taint	Whole meats	*Clostridium*, *Enterococcus*
Souring	Ham	Lactic acid bacteria, *Enterococcus*, *Micrococcus*, *Bacillus*, *Clostridium*

Source: Nychas *et al.* (2008)

Table 3. Factors and precursors affecting the production of odour end-products of Gram-negative bacteria, such as *Pseudomonas* spp., *Shewanella putrefaciens* and *Moraxella*

End product	Factors	Precursors
Sulfur compounds		
Sulfides	Temperature and substrate (glucose) limitation	Cysteine, cystine, methionine
Dimethylsulfide		Methanethiol, methionine
Dimethyldisulfite		Methionine
Methyl mercaptan		nad
Methanethiol		Methionine
Hydrogen sulfide	High pH	Cystine, cysteine
Dimethyltrisulfide	nad[a]	Methionine, methanothiol
Esters Methyl esters	Glucose (l)[b]	nad
Ethyl esters	Glucose (l)	nad
Aldehydes		
2-Methylbutanal	nad	*iso*-Leucine
Alcohols		
Methanol	nad	nad
Ethanol	nad	nad
2-Methylpropanol	nad	Valine
2-Methylbutanol	nad	*iso*-Leucine
Other compounds		
Ammonia	Glucose (l)	Amino acids

Adapted from Nychas *et al.* (2007)
[a] nad, no available data
[b] (l) low concentration of glucose

chilled meat (Holzapfel, 1998). These organisms have been isolated from beef carcasses during boning, dressing and chilling. Moreover, lairage slurry, cattle hair, rumen contents, walls of slaughter houses, the hands of workers, air in the chill room, neck and skin of the animal as well as the cut muscle surfaces have been shown to be contaminated with these organisms (Holzapfel, 1998; Nychas *et al*., 2008). Both LAB and *Br. thermosphacta* are the main, if not the most important, cause of spoilage, which can be recognized as souring rather than putrefaction (Table 2). *Br. thermosphacta* has been reported to be responsible for spoilage of meat products under refrigeration conditions (Lawrie and Ledward, 2006).

Need and forms of meat preservation in developing countries

Owning to the spoilage potential of meat, many varieties of preservation techniques are employed in improving its keeping quality and shelf life. In good hygienic conditions, after slaughter and evisceration, the optimal way to preserve meat is under refrigeration at temperatures around 4°C. However, in Nigeria and most African countries, because of lack of refrigeration facilities in the slaughter house, ambient temperatures above 20°C and lack of suitable transportation between the production and marketing areas, meat can be exposed to conditions of high risk with respect to increased contamination resulting from growth of pathogens and spoilage microorganism. Although most regulations recommend meat to be kept under refrigeration, the fact is that in many areas of most developing countries this does not occur (Guerrero *et al*., 1995).

In Nigeria, the majority of meat produced in abattoirs is sold for immediate consumption through retailers who buy from butchers and resell to consumers who usually subject it to cooking and consume within days. However, for various reasons, there are left-overs that are not sold. Since proper storage facilities are lacking, the left-over meat is processed into various forms in order to avoid

spoilage. This involves improvising traditional techniques of preservation. In cases where modern storage methods (such as refrigerators and freezers), are available, they are either expensive to maintain or means for their maintenance (electricity) are lacking. As an alternative, meat is preserved by processing to semi-dry and dry forms. A typical example is *kundi*, a dry meat product produced by cutting raw meat into pieces which is then parboiled and sundried in an open container, made of materials that can conduct heat. This method of preparation makes the meat product prone to microbial and other sources of contamination. However, the product, being an intermediate moisture meat (IMM), is low in moisture content and is shelf stable under tropical climates without refrigeration (Egbunike and Okubanjo, 1999).

Another product that meat is processed into is *Suya.* This is a popular, traditionally processed meat which is served or sold along streets, in club houses, on picnics and in restaurants. There are three main forms of *suya*, namely *tsire*, *kilishi* and *balangu*, but of these, *tsire* is the most commonly preferred (Alonge and Hiko, 1981). Therefore, to most consumers, *tsire* is synonymous with *suya* (Igene and Abulu, 1984). *Tsire* is a roasted, boneless meat of beef, goat or mutton that is cooked around a glowing charcoal fire in which the meat pieces are staked on wood sticks, spiced with peanut cake, spices, vegetable oil, salt or other flavourings. It is a delicatessen item since it does not receive any treatments designed to extend its shelf life (Harris *et al.*, 1975). Indeed, most sales-points hardly exhaust their sales and leftovers are often carried over to the second day or beyond. To this extent, rancidity often sets in, leading to the spoilage of this product. *Suya* products can become contaminated microbiologically from raw materials, handlers and/or equipment. Igene and Abulu (1984) reported the isolation of *Bacillus*, *Streptococcus*, *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas* and *Klebsiella* from raw and freshly roasted *tsire* subjected to different storage treatments. Uzeh *et al.* (2006) also reported the confirmation of some of these organisms in the stick meat, specifically *Ps. aeruginosa*, *B. cereus*, *Staph. aureus*, and *E. coli.*

As earlier noted, *Bacillus cereus* is one of the organisms that could cause food borne disease associated with consumption of contaminated meat. The genus, *Bacillus*, is also known to cause souring in meat (Table 1), while *B. anthracis* can cause disease in man, though regarded as a relatively low risk from meat and meat products (McClure, 2002). *B. cereus* is a ubiquitous organism and has been found in raw beef and milk, and the organism is directly linked to dairy cows. Therefore, contamination of carcasses of dairy cows is possible but is not thought to constitute a significant risk in foods of animal origin. Foodborne illness caused by *B. cereus* generally results from improper handling of foods (McClure, 2002). *Proteus* spp. have been found in small numbers in the flora on beef and pork carcases and in a variety of ready-to-eat processed meats (Nychas *et al.*, 2007). They have also been associated with the spoilage of beef; *Proteus* is known to be associated with putrefaction of meat (Nychas *et al.*, 2008).

Meat or meat products are not thought to be a major source of *Staph. aureus* as causative agent of food borne disease in man, even though the organism is an important pathogen in animals. The principal source of transmission between animals and man is unpasteurised milk and cheese made from unpasteurised milk (McClure, 2002). Outbreaks of staphylococcal food poisoning in man are frequently associated with improper food handling and temperature abuse of foods of animal origin, but it is generally believed that the main source of contamination is food handlers (Sofos, 2008). Nevertheless, strains of *Staph. aureus* can become endemic in food processing plants and meat can be contaminated from animal or human sources. *Staph. aureus* has been isolated from cattle carcasses and is also found in raw beef. The organism can become a major problem in cured meats as it is very salt tolerant and grows well when other flora are removed by the preservation methods.

Preservation using lactic acid bacteria

A general overview

The lactic acid bacteria comprise a group of Gram positive, non-sporulating, cocci or rods, and are catalase-lacking organisms. LAB produce lactic acid as the major end product during the fermentation of carbohydrates. They only grow in complex media where fermentable carbohydrates and higher alcohols are used as an energy source, mainly to form lactic acid. Homofermentative LAB degrade hexoses to lactate, whereas heterofermentative LAB degrade hexoses to lactate and additional products such as acetate, ethanol, CO_2, formate, or succinate. LAB are widespread in most ecosystems and are found in soil, water, plants, and animals. They are responsible for many food fermentation processes, but they are also commonly found on non-fermented foods such as dairy products, meat products, seafood, fruits, vegetables, cereals, sewage, and in the genital, intestinal, and respiratory tracts of humans and animals. LAB are widely used as protective cultures in the food industry for the production of fermented foods, including dairy (yogurt, cheese), meat (sausages), fish, cereals (bread and beverages such as beer), fruit (malolactic fermentation processes in wine production), and vegetables (sauerkraut, kimchi, silage).

Most LAB are considered as ‘generally recognized as safe’, GRAS (Silva *et al.* 2002). They are used to ensure safety, preserve food quality, develop characteristic new

flavours, and improve the nutritional qualities of food. LAB exert strong antagonistic activity against many related and unrelated microorganisms, including food spoilage organisms and pathogenic bacteria such as *Listeria, Clostridium, Staphylococcus* and *Bacillus* spp. The antagonistic effect of LAB is mainly due to a lowering of the pH of the food, to competition for nutrients, and to the production of inhibitory metabolites (Stiles, 1996). LAB are able to grow at refrigeration temperatures. They tolerate modified atmosphere packaging, low pH, high salt concentrations, and the presence of additives such as lactic acid, ethanol, or acetic acid.

The classification of LAB is based on morphological, metabolic and physiological criteria. As described earlier, LAB are related by a number of typical metabolic and physiological features. In the past few decades, DNA-based methods targeting genes such as 16S rRNA, applied to determine the relatedness of food-associated LAB, have resulted in significant changes in their taxonomic classification. The genera comprising LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*, as well as *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Teragenococcus*, *Vagococcus*, and *Weisella* (Stiles and Holzapfel, 1997). Members of the LAB typically have a G+C content below 50% (Stiles and Holzapfel 1997).

LAB in meat

In meats, LAB constitute a part of the initial microflora which develops easily after meat is processed to fermented sausages, chill stored or packed under vacuum or modified atmosphere. The strains of LAB generally considered as being found naturally in meats and meat products are: *Carnobacterium piscicola* and *C. divergens*; *Lactobacillus sakei*, *Lb. curvatus* and *Lb. plantarum*; *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuc. gelidum* and *Leuc. carnosum*. LAB in fresh meat bring about a mild fermentation process without producing any changes in the sensory characteristics because of the low carbohydrate content and the strong buffering capacity of meat. In the same way the growth of LAB in naturally fermented meats, after the addition of sugar, transforms the products through the production of lactic acid by the LAB. The subsequent decrease in pH denatures the meat proteins favouring the decrease of water activity (a_w), which ends up in a microbial stabilisation of the transformed product (Hugas, 1998).

In addition to the fermentable carbohydrates, glucose, glycogen, glucose-6-phosphate and small amounts of ribose, meat and meat products provide a number of vital growth factors such as available amino acids and vitamins that support the growth of the fastidious LAB. Some species of the genera *Lactobacillus*, *Carnobacterium*, *Leuconostoc* spp. and *Weissella* are especially well adapted to this ecosystem (Holzapfel, 1998). Several representatives of the genus *Lactobacillus* may typically dominate the microbial population especially of vacuum packaged and processed meat products. The facultative heteroferemtative species of *Lb. sake* and *Lb. curvatus* are found in most meat systems and are probably the most frequently encountered species of the genus. These two species have been shown to be of major economic importance in meat products, or acting as main and desirable fermentative organisms in dry sausages (Holzapfel, 1998; Conter *et al*., 2005).

Persistence and competitive ability of *Lactobacillus* and several other species of the genera *Leuconostoc* (*Leuc. amelibiosum*, *Leuc. carnosum*, *Leuc. gelidum*), *Weissella* (*W. viridescens*, *W. halotolerans*) and *Carnobacterium* (*Cb. divergens*, *Cb. piscicola*) in processed meat systems are explained by their ability to ferment the carbohydrates in meat and their adaptation to the meat substrate. While the leuconostocs appear to grow most rapidly on chilled fresh meat (Borch and Agerhem, 1992), *Lb. curvatus* and *Lb. sake*, on account of their higher tolerance of elevated salt concentrations and nitrite, typically dominate raw fermented sausage and pasteurized emulsified meat products (Holzapfel, 1998). Some of these features also apply to two species of the genus *Pediococcus*, *Ped. pentosaceus*, and *Ped. acidilactici*, which are associated with fermented meat products (Albano *et al*., 2007).

Enterococcus and *Lactococcus* are other genera of LAB that are of some commercial significance. *Enterococcus* spp. use the homolactic pathway for energy production, yielding mainly L(+) lactic acid from glucose at pH values less than 5. At pH values above 7, ethanol, acetic acid and formic acid are the main products of glucose fermentation. In the absence of heme and under aerobic conditions, glucose is converted to acetic acid, acetoin and carbon dioxide. The genus *Enterococcus* differs from the lactococci by their resistance to 40% bile and growth of most species at 6.5% salt. *E. faecium* and *E. faecalis* are associated with the gastro-intestinal tract of man and warm-blooded animals and have been suggested as indicators of faecal contamination of meat (Franz *et al*., 1999).

The association of pediococci with proteinaceous foods such as fresh and cured meat, and raw sausages, has frequently been reported and particularly for *Ped. acidilactici* and *Ped. pentosaceus* in fermented sausages (Porubcan and Sellars, 1979; Onilude *et al*., 2002; Conter *et al*., 2005; Albano *et al*., 2007; Olaoye *et al*., 2008; Olaoye and Onilude, 2009). The association of pediococci with meat fermentations has been a topic of intensive study (Holzapfel, 1998; Albano *et al*., 2007). Meat and meat products provide a favourable growth substrate for strains of *Ped. acidilactici* and *Ped. pentosaceus*, and particularly in the fermentation of semi-dry sausages or other cured products, such strains appear to play some role during fermentation and

maturation (Parente *et al*., 2001). Pediococci are also frequently found in vacuum or modified-atmosphere-packaged meat and meat products, in which the LAB population is, however, most often dominated by species of the genera *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Weissella* and *Enterococcus* (Jones, 2004).

Use of LAB as biological preservatives

Biopreservation, preservation by the use of biological agents, refers to the extension of the shelf-life and improvement of the safety of foods using microorganisms and/or their metabolites (Ross *et al*., 2002). Antagonistic cultures which are added to meat products to inhibit pathogens and/or prolong the shelf life, while changing the sensory properties as little as possible, are termed protective cultures (Lucke, 2000). Their antagonism refers to inhibition through competition for nutrients and/or production of one or more antimicrobially active metabolites (Table 4; Holzapfel *et al*., 1995). In a recent study by Olaoye and Onilude (2010), the potential of selected species of *Pediococcus* as biological preservatives in the extension of shelf life of fresh beef in Nigeria was investigated. The authors reported that the LAB strains used were able to effect preservation of the meat product, for few days before spoilage was started to set in. In a similar study, Olaoye and Dodd (2010) also reported the extension in shelf life of *tsire*, a traditional Nigerian stick meat, after treatment with bacteriocinogeic cultures of *Pediococcus*.

Nowadays, the consumer pays a lot of attention to the relation between food and health. As a consequence, the market for foods with health-promoting properties, so called functional foods, has shown a remarkable growth over the past few years (Leroy and De-Vuyst, 2004). Also, the use of food additives is regarded as unnatural and unsafe (Ray, 1992). Yet, additives are needed to preserve food products from spoilage and to improve the organoleptic properties; hence the use of functional protective cultures in the food fermentation industry is being explored. Functional protective cultures are microorganisms that possess at least one inherent functional property. The latter can contribute to food safety and/or offer one or more organoleptic, technological, nutritional, or health advantages. The implementation of carefully selected strains as microbial cultures or co-cultures in fermentation processes can help to achieve *in situ* expression of the desired property, maintaining a perfectly natural and healthy product. Examples are LAB that are able to produce antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals, or LAB with health-promoting properties, so called probiotic strains. This represents a way of replacing chemical additives by natural compounds, at the same time providing the consumer with new, attractive food products and it also leads to a wider application area and higher flexibility of cultures (Jahreis *et al*., 2002; Pidcock *et al*., 2002).

Production of antimicrobials by LAB for food preservation

In meat, production of one or more antagonistic metabolites may be part of the complex mechanism by which a micro-organism becomes established in the presence of other competing organisms (Holzapfel, 1998). The understanding of such mechanisms provides a valuable key to our understanding the complexity of microbial interactions in a meat system and hence the basis of 'biological' approaches to food preservation. One of the main roles of LAB in biopreservation is to improve safety by inactivating pathogens and spoilage microorganisms via acid production and bacteriocins. Furthermore, it is essential that potential biopreservative cultures show no pathogenic or toxic activities (Hammes and Hertel, 1996; Ammor and Mayo, 2007). The food industry is expected to produce safe, healthy and nutritious products of high quality. For many food products, fermentation with starter cultures containing lactic acid bacteria (LAB) is an essential part of the production process.

Organic acid production

An important role of meat LAB starter cultures is the rapid production of organic acids; this 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. The lipophilic nature of the undissociated acid allows it to diffuse across the cell membrane collapsing the electrochemical proton gradient. Alternatively, cell membrane permeability may be affected, disrupting substrate transport systems (Snijders *et al*., 1985). The types and levels 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).

Fermentation of the carbohydrates, glucose, glycogen, glucose-6-phosphate and small amounts of ribose, in meat and meat products, produces organic acids by glycolysis (Embden-Meyerhof Parnas pathway, EMP-pathway; Figure 1) or the Hexose Monophosphate, HMP-pathway. L (+) lactic acid is more inhibitory than its D(-) counterpart (Benthin and Villadsen, 1995). Lactic acid is a major fermentation end product of LAB and a number of other genera (e.g *Brochothrix*). The LAB in particular

Table 4 Metabolic products of lactic acid bacteria with antimicrobial properties

Product	Main target organisms
Organic acids	
Lactic acid	Putrefactive and Gram-negative bacteria, some fungi
Acetic acid	Putrefactive bacteria, clostridia. some yeasts and fungi
Hydrogen peroxide	Pathogens and spoilage organisms. especially in protein-rich foods
Low-molecular-weight metabolites	
Reuterin (3-OH-propionaldehyde)	Wide spectrum of bacteria, moulds and yeasts
Diacetyl	Gram-negative bacteria
Fatty acids	A range of different bacteria
Bacteriocins	
Nisin	Some LAB and Gram-positive bacteria, notably endospore-formers
Others	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

Source: Holzapfel *et al*. (1995).

Table 5 Classification of bacteriocins from lactic acid bacteria

Category	Subcategory
Class I—lantibiotics	Type A: elongated molecules Subtype A1: leader peptides are cleaved by a dedicated serin proteinase Subtype A2: leader peptides are cleaved by a dedicated ABC- transporter Type B: globular molecules
Class II—nonmodified, heat-stable bacteriocins	Class IIa: pediocin-like bacteriocins Class IIb: two-peptide bacteriocins Class IIc: *sec*-dependent bacteriocins Class IId: other bacteriocins
Class III—large, heat-labile bacteriocins	

Source : Nes *et al*. (1996) and Moll *et al*. (1999)

are able to reduce the pH to levels where putrefactive (e.g. clostridia and pseudomonads), pathogenic (e.g. salmonellas and *Listeria* spp.) and toxinogenic bacteria (*Staphylococcus aureus*. *Bacillus cereus*, *Clostridium botulinum*) will be either inhibited or killed (Holzapfel *et al*., 1995; Holzapfel, 1998). Also, the undissociated acid, on account of its fat solubility, will diffuse into the bacterial cell, thereby reducing the intracellular pH and slowing down metabolic activities, and in the case of Enterobacteriaceae such as *E. coli* inhibiting growth at around pH 5.1. The rapid reduction of the pH below 5.3 during sausage fermentation is sufficient to inhibit growth of salmonellas and *Staph. aureus* (Holzapfel, 1998).

Bacteriocin production by LAB

The bacteriocins of LAB possess common traits that justify their classification on a sound scientific basis into three well defined classes (Nes *et al*., 1996; Moll *et al*., 1999) (Table 5):

Class I, the lantibiotics, small heat-stable polycyclic peptides (<5 kDa) containing small, membrane active

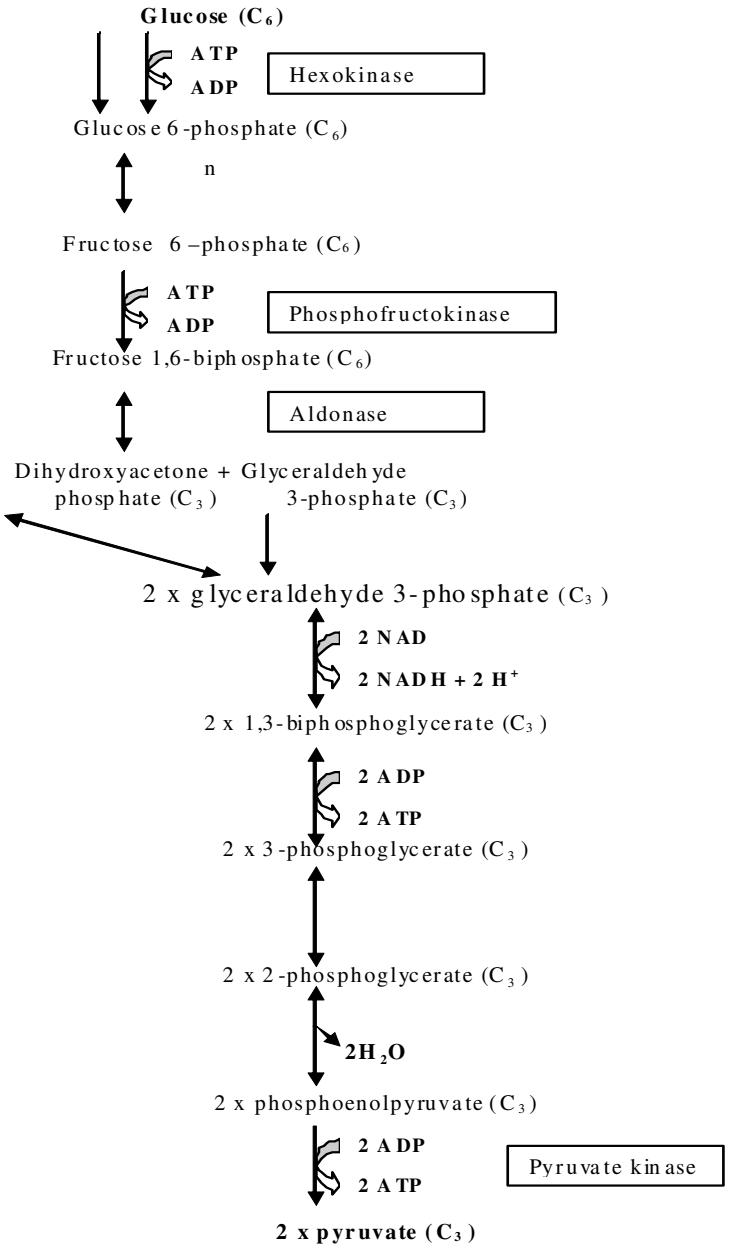

Figure 1. Embden Meyerhof Parnas pathway
Source: Adam and Moss, 2008

peptides;
Class II, the small (<10 kDa) heat-stable non-lantibiotics such as pediocin-like bacteriocins with a strong anti-Listeria activity;
Class III, large (>30 kDa) heat-labile bacteriocins.
Due to their abundance and possible application in industrial processes, bacteriocins belonging to the first two classes are the most thoroughly studied (Nes *et al*., 1996; Moll *et al*., 1999). The most prominent Class I bacteriocin is nisin, which is produced by strains of *Lactococcus lactis* subsp. *lactis* isolated from milk and vegetable-based products (Harris *et al*., 1992) and by *Lc. lactis* BB24 isolated from Spanish-dry fermented sausages (Rodríguez *et al*., 1995; Cintas *et al*., 1998). Nisin is a broad spectrum bacteriocin with bactericidal activity towards a wide range of Gram-positive bacteria, including *Staphylococcus aureus* and *Lis. monocytogenes* (Cintas *et al*., 1998). In addition, nisin prevents spore outgrowth and inhibits vegetative cells of *Bacillus* spp. and *Clostridium* spp. (Abee *et al*., 1995). To

date, nisin is the most thoroughly studied and characterized bacteriocin of LAB and the only one internationally accepted as a food biopreservative in certain foods (Delves-Broughton *et al*., 1996).

Class II bacteriocins (non-lantibiotics) comprise a heterogeneous group of bacteriocins. Despite differences in their primary structures, most Class II bacteriocins are small (<10 kDa) and heat-stable peptides with a high content of small amino acids such as glycine.

They are usually cationic and often amphiphilic, reflecting their ability to kill target cells by permeabilizing the cell membrane (Nes *et al*., 1996; Moll *et al*., 1999). Class IIa bacteriocins are the most thoroughly studied LAB bacteriocins and possess interesting technological properties and a strong antimicrobial activity against a broad range of Gram-positive spoilage and food-borne pathogens, especially *Lis. monocytogenes*. The search for LAB producing antilisterial bacteriocins has lead to the description and characterization of a large number of Class IIa bacteriocins, produced by a wide variety of *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactobacillus* and *Carnobacterium* strains. Class IIa is also referred to as the pediocin family, which is named from pediocin PA-1, the first and most thoroughly characterized bacteriocin within the group (Marugg *et al*., 1992; Nieto-Lozano *et al*., 1992). Pediocin-like bacteriocins, members of the class II bacteriocins, are of considerable commercial interest owing to their characteristics of being small, heat-resistant peptides that are not modified post-translationally. All the pediocin-like bacteriocins share certain features, including a seven amino acid conserved region in the N-terminal of the active peptide (–Tyr–Gly–Asn–Gly–Val–Xaa–Cys–; Ennahar *et al*., 2000). They are active against other LAB but are particularly effective against *Lis. monocytogenes* (Calo-Mata *et al*., 2008). Pediocin PA-1 is, perhaps, the best known, produced by *Pediococcus acidilactici* isolated from American-style sausages and *Ped. pentosaceus* Z102 from Spanish style sausages (Castellano *et al*., 2008; Calo-Mata *et al*., 2008). In the past, several pediocin PA-1-producing LAB strains were independently isolated in different laboratories (Bennik *et al*., 1997; Rodríguez *et al*., 1997). However, in many cases the bacteriocin produced received different names (pediocins PA-1, AcH, JD, Bac and 347, mesentericin 5) before identification and realization that all were the same molecule (Rodríguez *et al*., 2007). The pediocin PA-1-containing fermentate Alta^{TM} 2341 is a commercial food ingredient reported to extend the shelf life of a variety of foods and, particularly, to inhibit the growth of *Lis. monocytogenes* in ready-to-eat meat products (Rodríguez *et al*., 2007). The determination of the pediocin PA-1 amino acid sequence, the application of improved protocols for its purification, and the identification of the pediocin PA-1 operon have been reported (Nieto-Lozano *et al*., 1992; Marugg *et al*., 1992).

The high molecular weight Class III bacteriocins have been identified within the genera *Lactobacillus* and *Enterococcus* (Fremaux and Klaenhammer, 1993). These bacteriocins, in contrast to Class I and II bacteriocins, are inactivated upon heat treatment (e.g., 60 - 100 ºC for 10 - 15 min) and, similar to type B lantibiotics, do not act on sensitive cells by membrane-disruption.

Interest in the bacteriocins produced by meat LAB has increased dramatically, reflecting their growing importance with respect to the functional properties of starter cultures (Abee *et al*., 1995). A number of bacteriocins are produced by most LAB species involved in meat fermentation, including *Lb. sakei, Lb. curvatus, Lb. plantarum*, and *Ped. acidilactici* (Enan *et al*., 1996). Meat-borne LAB produce a range of bacteriocins that are generally active towards other LAB (contributing to the competitiveness of the producing strain) and food borne Gram–positive pathogens such as *Lis. monocytogenes*, *Staph. aureus*, *C. perfringens* and *B. cereus* (Noonpakdee *et al*., 2003). Bacteriocins exert their inhibitory action via the formation of pores in the cytoplasmic membrane of sensitive cells as well as interrupting DNA and protein syntheses (Calo-Mata *et al*., 2008). Generally, bacteriocins target the cell envelope and, with the exception of the larger proteins (>20 kDa) that degrade the murein layer (e.g. lysins and muramidases), use non-enzymatic mechanisms to cause the depolarization of the target cell membrane and/or inhibit cell wall synthesis (Settanni and Corsetti, 2008). Bacteriocins have generally a cationic character and easily interact with Gram-positive bacteria that have a high content of anionic lipids in the membrane determining the formation of pores (Chen and Hoover, 2003). Pores in the cytoplasmic membrane clearly affect the energetic status of the cell, i.e. dissipation of proton motive force (PMF) causing an arrest of ΔpH and $\Delta\psi$ (transmembrane electrical potential) dependent processes (such as transport) while certain bacteriocins cause ATP efflux (Settanni and Corsetti, 2008). A bacteriocin producer protects itself against its own antimicrobial compound by means of a system referred to as immunity, which is expressed concomitantly with the antimicrobial peptide (Nes *et al*., 1996; Settanni and Corsetti, 2008). The mode of action of bacteriocins can be bactericidal or bacteriostatic, determining death or extension of lag phase respectively. In Gram-positive bacteria, the bacteriocin nisin produced by *Lc. lactis* has been shown to act on energized membrane vesicles to disrupt PMF, inhibit uptake of amino acids, and cause release of accumulated amino acids (Jack and Tagg, 1991). Studies on the mode of action of bacteriocins have indicated that bactericidal activity was confined to pH values of 6 and lower (Abee *et al*., 1995). This is possibly due to the influence of two positively-charged (lysine) and two negatively-charged (glutamate and aspartate) amino acids and two histidine residues with a positive charge at pH 6 or lower (pK_a = 6 for His) and having a major role in determining the effective charge of the peptide which is

crucial for activity (Abee *et al*., 1995). 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 meat products than in *in vitro* systems. Activity may be reduced by the binding of the bacteriocin molecules to food components (mainly the fat matrix), and by the destabilizing action of proteases and other enzymes (O'Keeffe and Hill, 2000). Further limitations of bacteriocin effectiveness are uneven distribution in the food matrix and their inhibition by salt and curing agents (Leroy and de Vuyst, 1999; O'Keeffe and Hill, 2000; Calo-Mata *et al*., 2008). Even so, several authors report that certain bacteriocinogenic meat LAB could be used as bioprotective cultures to prevent the growth of pathogens in sausage. Indeed, the use of bacteriocin-producing *Lactobacillus sakei* as a starter culture decreases the numbers of *Listeria* in fermented sausage (De Martinis and Franco, 1998). Antilisterial effects have also been demonstrated with bacteriocinogenic *Lb. curvatus*, *Lb. plantarum* and *Ped. acidilactici* (Luchansky *et al*., 1992; Dicks *et al*., 2004). The production of bacteriocins with a broad inhibition range, especially towards food-borne pathogens is therefore highly desirable since this would ensure the competitiveness of the starter strain while reducing the numbers of harmful flora.

Other antimicrobials of LAB

Hydrogen peroxide is produced from lactate by LAB in the presence of oxygen as a result of the action of flavoprotein oxidases or nicotinamide adenine dinucleotide (NADH) peroxidise (Ammor and Mayo, 2007). The antimicrobial effect of H_2O_2 may result from the oxidation of sulfhydryl groups causing denaturing of a number of enzymes, and from the peroxidation of membrane lipids thus increasing membrane permeability (Kong and Davison, 1980). H_2O_2 may also be a precursor for the production of bactericidal free radicals such as superoxide (O^{-2}) and hydroxyl (OH^-) radicals which can damage DNA (Byczkowski and Gessner, 1988). The enzyme catalase hydrolyses hydrogen peroxide. Some LAB strains involved in meat fermentation, such as *Lb. sakei*, *Lb. plantarum*, *Lb. pentosus* and *Ped. acidilactici*, possess heme-dependent catalase activity which is active in meat products since these substrates contain heamin in abundance (Abriouel *et al*., 2004; Ammor *et al*., 2005). Most undesirable bacteria such as *Pseudomonas* spp. and *Staph. aureus* are many times more sensitive than the LAB to H_20_2.

Carbon dioxide is mainly produced by heterofermentative LAB. The precise mechanism of its antimicrobial action is still unknown. However, CO_2 may play a role in creating an anaerobic environment which inhibits enzymatic decarboxylations, and the accumulation of CO_2 in the membrane lipid bilayer may cause a dysfunction in permeability (Eklund, 1984). CO_2 can effectively inhibit the growth of many food spoilage microorganisms, especially Gram-negative psychrotrophic bacteria (Farber, 1991). The degree of inhibition by CO_2 varies considerably between the organisms. CO_2 at 10% (v/v) could lower the total bacterial counts by 50% (v/v) (Wagner and Moberg, 1989), and at 20–50% it had a strong antifungal activity (Lindgren and Dobrogosz, 1990). Pathogens (e.g Enterobacteriaceae and *Listeria*) could also be inhibited due to reduced pH effects as CO_2 dissolves to produce a weak acid.

Diacetyl, an aroma component, is produced by strains within all genera of LAB by citrate fermentation. It is produced by heterofermentative lactic acid bacteria as a by-product along with lactate as the main product. Diacetyl is a high value product and is extensively used in the dairy industry as a preferred flavour compound. *Lb. rhamnosus* gives a high yield for diacetyl, 64 mg of diacetyl per g of glucose consumed (Anuradha *et al*., 1999). The physiological reason for the production of diacetyl is not clearly understood. It is hypothesized that diacetyl is synthesized to reduce the toxicity of pyruvate. Diacetyl also has antimicrobial properties. It inhibits the growth of Gram-negative bacteria by reacting with arginine utilization (Jay, 1986). Jay (1982) showed that Gram-negative bacteria were more sensitive to diacetyl than Gram-positive bacteria; the former were inhibited by diacetyl at 200 µg/ml. The antimicrobial activity of diacetyl was evaluated against *E. coli*, *Lis. monocytogenes* and *Staph. aureus* in a study by Lanciotti *et al.* (2003); the authors concluded that the organisms were sensitive to diacetyl with *Lis. monocytogenes* having the least susceptibility. Generally varying concentrations of diacetyl are required to bring about inhibitions of different pathogenic and spoilage organism (Lanciotti *et al*., 2003).

Beneficial effects of LAB on meat

As noted earlier in this report, strains of LAB to be used in the biopreservation must be carefully selected in order to achieve the desired beneficial effect. This is because not all LAB cultures can be used to achieve the purpose. The use of LAB as biological preservatives on meat products could confer health benefits to the consumers. A comprehensive note has been reported by Olaoye and Idowu (2010) on the various features and properties of LAB used as biological preservatives of meat processing. According to the authors, 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.

CONCLUSION

In conclusion, spoilage of meat is inevitable, especially in developing countries where storage systems have been very epileptic. Although, in such countries, meat is being processed into other forms to avoid the associated spoilage, the potential of lactic acid bacteria as biological preservatives could be exploited in complementing the existing traditional preservation techniques.

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