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Review

Research progress in the use of lactic acid bacteria as natural biopreservatives against *Pseudomonas* spp. in meat and meat products: A review

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ABSTRACT

Meat and meat products represent excellent sources of key nutrients for human health, such as protein, essential amino acids, B vitamins, and minerals. However, they are recognized as highly perishable foods since they represent an ideal substrate for the growth of spoilage and pathogenic microorganisms. Meat spoilage is a complex process that involves multiple microorganisms and a combination of intrinsic and extrinsic ecological factors. One of the most common causative agents of meat spoilage is represented by species of the genus *Pseudomonas*. To prevent the development of such undesired microorganisms, chemical preservatives are usually exploited by the meat industry. However, the growing consumers' concerns about potential health issues linked to the consumption of chemical preservatives has prompted the food industry to develop alternative strategies to prevent microbial spoilage in meat and meat products. Besides to the application of physical strategies, the interest towards the use of natural preservatives, such as bioprotective microorganisms (e.g., lactic acid bacteria) and their metabolites, has rapidly grown. When used in meat and meat-based products, lactic acid bacteria exhibited a bioprotective activity against spoilage and even foodborne pathogens, thanks to the production of different inhibitory compounds including organic acids, bacteriocins, carbon dioxide, hydrogen peroxide, ethanol, N-diacetyl, and lactones. This bioprotective activity might justify the use of lactic acid bacteria or their metabolites as natural preservatives to extend the shelf-life of the products. However, the effectiveness of antimicrobial activity against *Pseudomonas* in meat and meat products still needs to be investigated to understand the influence of the type of end product, the type of packaging, and the storage conditions (time and temperature). Moreover, the antimicrobial activity of lactic acid bacteria must also be evaluated taking into consideration their ability to maintain the sensory features of fresh meat (whether whole or minced), without negatively affecting its sourness and acidity. Of note, the results herein discussed emphasize the challenges occurred in translating *in vitro* findings into practical applications due to the complex interactions between bacteria, antimicrobial compounds, and food matrices.

1. Introduction

Meat and meat products have always been part of human's diet, thanks to their high content in nutrients such as high-quality protein, lipids, vitamins, and minerals. However, meat also represents an important source of nutrients for spoilage and pathogenic foodborne microorganisms, which can easily grow, causing an increase in food waste and economic losses for both the meat industry and consumers (Woraprayote et al., 2016). In more detail, meat spoilage is caused by the so-called specific spoilage organisms (SSOs), which can dominate the meat environment, producing metabolites that negatively affect the quality of meat (color, texture, appearance, and flavor) and making it undesirable and unfit for human consumption (Lianou et al., 2016; Das et al., 2019; Biswas et al., 2021). The SSOs group includes *Pseudomonas* spp., lactic acid bacteria, *Brochothrix thermosphacta*, Enterobacteriaceae, *Acinetobacter* spp., *Aeromonas* spp., *Alcaligenes* spp., *Moraxella* spp., *Flavobacterium* spp., *Staphylococcus* spp., and *Micrococcus* spp. (Ercolini et al., 2009, Mutwakil, 2011, Mellor et al., 2011, Wang et al., 2017).

Contamination of meat can start during the pre-slaughter phases and continue throughout the slaughtering process of animals. The pre-

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slaughter phases are represented by feed withdrawal at farm, catching and crating, transport of living animals, and lairage at slaughtering plant. These phases influence the contamination levels of the carcasses, due to possible *ante-mortem* fecal contamination, resulting in poor hygienic conditions. The slaughtering phases include stunning and killing, bleeding, evisceration, and chilling. During slaughtering, the carcasses can be contaminated through contact with animal skin, intestinal content, and equipment. As reported by Song et al. (2021), during slaughtering microbial load of *Pseudomonas fluorescens*, *Pseudomonas fragi* and *Salmonella enterica* can reach values of 3 log (cfu/g) in fresh chicken meat, whereas, on the surfaces of equipment, *S. enterica* can reach loads of 2–4 log (cfu/cm^2) (Russo et al., 2006). During slaughtering, the evisceration phase can be crucial in determining the contamination of animal carcasses from fecal bacteria which reside in the gastrointestinal tract of the animal, such as Enterobacteriaceae, including *Escherichia coli* and coliforms. Slaughterhouse operators can also contribute to microbial contamination of carcasses and meat due to a lack in hygiene practices. In more detail, the main bacteria related to human cross-contamination of carcasses are staphylococci, with particular attention to *Staphyloccus aureus* (Bencardino et al., 2021; Rani et al., 2023). The degree of contamination reached during these early phases of meat processing is crucial to determine the spoilage susceptibility of fresh meat and meat products. In fresh meat, bacteria are located on the surface, whereas in the processed product (cured, minced, or reconstructed meat) they can penetrate the muscle (Odeyemi, Alegbeleye, Strateva, & Stratev, 2020).

Other than pre-slaughtering and slaughtering phases, other factors contribute to increasing spoilage of meat and meat products, such as sudden changes in temperature, pH, water activity (a_w), moisture content, poor sanitation of processing machineries, and inadequate packaging and storage conditions.

In more detail, handling and storage temperatures are key factors in inducing meat spoilage. High temperatures speed up the degradative process, promoting the growth of both bacteria and molds, whereas refrigeration temperature determines a selection of specific microorganisms such as psychrotrophic bacteria like *Pseudomonas* spp. and *B. thermosphacta* (Mellor et al., 2011; Wang et al., 2017).

Meat pH is also an important factor affecting the spoilage process: normal post-mortem pH of meat varies from 5.4 to 5.9 and it can be affected by pre-slaughter stress of the animal. Stressed animals can result in the expression of abnormalities such as dark firm dry (DFD) meat in chicken, which is caused by chronic pre-slaughter stress (e.g., fatigue and long fasting) and results in high pH values (>6) in chicken meat, speeding up the microbial spoiling process. Indeed, high pH values result in rapid meat spoilage, due to consumption of nutrients, hydrolyzation of amino acids, and production of catabolites by growing microorganisms.

Water activity (a_w) and moisture content also play a crucial role in meat spoilage. Fresh meat has a moisture content proximal to 75 % and reaches a_w value of 0.99, making it prone to microbial growth. The addition of different ingredients, such as sodium chloride, during the manufacturing process of meat products, can lower a_w values to 0.95–0.96, thus slowing down the degradative process. Indeed, most microorganisms need a_w values ranging from 0.97 to 0.99. Borch et al. (1996) showed that a decrease of the a_w value from 0.99 to 0.97, using 4 % sodium chloride, inhibited the growth of salt-sensitive microorganisms (e.g., *Pseudomonas* spp. and Enterobacteriaceae) and prolonged the lag phase of salt-tolerant microorganisms such as lactic acid bacteria.

Packaging and storage conditions also influence microbial spoilage of meat and meat products, selecting the type of microorganisms that will grow during product's shelf life. Indeed, the presence or absence of oxygen has a strong impact on the microbial species that can develop in the food matrix, therefore, the type of packaging conditions (modified atmosphere packaging – MAP, vacuum packaging, or aerobic packaging) can affect the final quality of the meat product. In more detail, modified packaging atmosphere can play a crucial role in influencing the growth of different microbial species, depending on the gas ratio. Of note, the presence of CO_2 has an antimicrobial effect thanks to its acidifying effect, whereas the presence of high ratios of N_2 instead of oxygen inhibits the growth of aerobic species. In vacuum packed meat and meat products, the absence of oxygen acts in selecting anaerobic and facultative anaerobic microorganisms such as clostridia and lactic acid bacteria (Erkmen & Bozoglu, 2016). The standard aerobic packaging protects the product from external contamination but does not play any role in influencing the growth of microorganism already present in meat and meat products. Under aerobic packaging, *Pseudomonas* spp. is, in most cases, responsible for meat and meat products spoilage (Erkmen & Bozoglu, 2016), even though some studies have shown the ability of species of the *Pseudomonas* genus to grow in vacuum packaged deli meats (Bower et al., 2017; Furbeck et al., 2022).

In the present review, the main features of *Pseudomonas* spp. as spoilage microorganisms in meat and meat products are presented, together with the conventional strategies currently applied by the meat industry for preventing meat spoilage by this microbial genus. Then, recent advancements in using lactic acid bacteria as natural biopreservatives against *Pseudomonas* spp. in meat and meat products are presented and discussed. This will provide the meat industry with novel strategies capable of potentially reducing meat spoilage through natural means.

2. *Pseudomonas* spp. as spoilage microorganisms in meat and meat products

Pseudomonas is a bacterial genus of the Pseudomonadaceae family that contains more than 140 species, most of which are saprophytic. *Pseudomonas* species are aerobic/facultative anaerobic, non-spore-forming, oxidase-positive, catalase-positive, Gram-negative, rod-shaped, respiratory bacteria characterized by the presence of one or several flagella and pili (Iglewski, 1996; Palleroni & Moore, 2004). Most of the *Pseudomonas* species are psychrotrophic, being able to grow at temperatures below 7 °C, with a temperature range of 0–40 °C (Guillou & Guespin-Michel, 1996; Elbehiry et al., 2022; Xing et al., 2023), and being the causative agents of spoilage of foods stored under aerobic conditions at low temperatures, such as meat, meat products, milk, dairy products, and fish (Nychas et al., 2008; Ercolini et al., 2010; Mellor et al., 2011; Ahn et al., 2012; Bruckner et al., 2012; Remenant et al., 2015; Hutchings et al., 2021). In more detail, the species *P. fluorescens*, *P. fragi*, *Pseudomonas lundensis*, *Pseudomonas migulae*, and *Pseudomonas putida* have frequently been isolated from spoiled chilled meat (Ercolini et al., 2006; Doulgeraki et al., 2012), being responsible for gross discoloration, greening, slime, and malodor generation (e.g., putrid and sulphury odors), due to the production of dimethyl sulfide (Borch et al., 1996; Labadie, 1999; Casaburi et al., 2015; Zagorec & Champomier-Vergès, 2017; Wickramasinghe et al., 2019).

Due to the high adaptability of *Pseudomonas* species to the meat environment, sanitation phases, hygiene of processing, and operator awareness of good manufacturing practices (GMP) play a crucial role in spoilage prevention. Previous studies carried out by Caldera et al. (2016) and Raposo et al. (2017) have demonstrated that pseudomonads, in meat and meat products, have the ability to release thermotolerant proteolytic and lipolytic enzymes, thus helping *Pseudomonas* spp. survival and growth, and determining serious quality and shelf-life decrease (Nychas et al., 2008; Mellor et al., 2011; Casaburi et al., 2015). *Pseudomonas* species and biotypes isolated from meat and meat products have often been characterized by the ability of biofilm formation on production surfaces and by resistance to standard cleaning procedures (Grobe et al., 2001; Wirtanen et al., 2001; Giaouris et al., 2015; Stellato et al., 2017). The presence of *Pseudomonas* spp. in meat is also considered to be linked to the generation of a microaerophilic environment that can help the survival of aerobic pathogens, such as *Campylobacter jejuni* (Hilbert et al., 2010; Morales et al., 2016). Despite having poor nutritional requirements, *Pseudomonas* spp. require high a_w

values, above 0.97 (Dagorn et al., 2013; Dimassi et al., 2020), and pH values from 5.6 to 7.1 (Lin et al., 2016). These environmental conditions can be easily found in meat and meat products, in which several studies reported the growth of *Pseudomonas* species during the storage period. In more detail, Bruckner et al. (2012) observed that in pork loin chops packed under aerobic packaging and stored at 4 °C, *Pseudomonas* spp. initial counts had a mean value of 3.5 log CFU/g, reaching values of 9–10 log CFU/g after 8 days of storage. The same authors also evaluated the growth of *Pseudomonas* spp. in chicken breast fillets, observing initial counts of 4.10 log CFU/g and final counts of 9–10 log CFU/g after 8 days of storage at 4 °C under aerobic packaging (Bruckner et al., 2012). Similar values were detected by Mellor et al. (2011) in chicken skin aerobically stored at 4 °C. Despite being recognized as an aerobic group of microorganisms, Doulgeraki and Nychas (2013) observed that, in minced beef, pseudomonads were able to grow and reach high counts also in meat under MAP (40 %CO_2 – 30 %O_2 – 30 %N_2), detecting the growth of *P. fluorescens*, *P. putida*, and *P. fragi*.

3. Strategies for preventing meat spoilage by *Pseudomonas* spp.

Meat industry has been putting a lot of efforts to contrast the development of spoilage microorganisms as *Pseudomonas*. During years, different preventive methods have been discovered and applied to meat and meat products, resulting in the inhibition of undesired spoilage bacteria (including *Pseudomonas*).

3.1. Chemical methods

The cheaper and most applied preservation method applied by the meat industry is usually represented by the addition of chemical preservatives. In more detail, organic acids and derivatives such as acetic, lactic, propionic, and citric acids and their salts (Stanojević-Nikolić et al., 2016; FSIS, 2018), and nitrites and nitrates (Keto-Timonen et al., 2012; Govari & Pexara, 2018) are typically used in meat processing in order to ensure microbiological safety and stability of the final product. However, during the last decades, the growing consumers' concern about potential health issues linked to the consumption of chemical preservatives has prompted the food industry to develop alternative strategies to prevent microbial spoilage in meat and meat products. In fact, several papers reported cases of mutagenic and carcinogenic effects related to sorbic acid and its salts (which are commonly used as chemical preservatives), whereas other authors stressed the connections between nitrates and nitrites consumption and leukemia, colon cancer, and bladder cancer (Lee and Paik, 2016; Crowe et al., 2019). Due to these findings, consumers have become more interested in knowing the list of ingredients reported in the product's label, avoiding foods with added chemical preservatives (E- numbers). It is important to mention that chemical preservatives' addition in meat products and in meat preparations is strictly regulated by the EU Regulation (EC) No 1333/2008 of the European Parliament and of the Council, which allows or prohibits the addition of specific chemical preservative and sets specific dosage levels in the different meat products.

3.1.1. Thermal processing methods

Apart from chemical preservatives, thermal processing is one of the most common preservation methods employed by the meat industry (e.g., canned meat, cooked ham, coated-fried products, etc.) and is based on heating food at a certain temperature for a certain time of exposure (Hassoun et al., 2021). The combination of time and temperature implicates the elimination, or the reduction to acceptable levels, of the *Pseudomonas* spp. cells. However, to ensure microbiological stability and safety of the treated product, thermally processed foods are often overheated (Wold et al., 2020; Hassoun et al., 2021). The overtreatment of the product may result in unwanted sensory and nutrient loss, such as protein denaturation, fat oxidation, and vitamin loss (Hassoun et al., 2021).

3.1.2. Use of modified atmosphere

While thermal treatment causes a bactericidal effect, other treatments, such as MAP, result in a bacteriostatic effect. MAP consists in a food packaging method where the air within a package is replaced by a mixture of different gases before the sealing step (Stammen et al., 1990). In meat and meat products, the aim of MAP is to modify the gas composition in the package headspace, in order to prevent and/or delay unwanted chemical reactions and the growth of spoilage microorganisms, including *Pseudomonas* spp. (Tsironi et al., 2019). Aerobic bacteria like *Pseudomonas* spp. are inhibited by CO_2, therefore MAP applied to meat and meat products is usually composed by 20–30 % CO_2 and 70–80 % O_2 in order to extend the shelf life of the products (Kennedy et al., 2005; Soldatou et al., 2009; Lindahl et al., 2010; Piergiovanni & Limbo, 2010; Arvanitoyannis & Stratakos, 2012; Papuc et al., 2017).

3.1.3. Non-thermal processing methods

Novel methodologies to avoid loss of nutrients are represented by non-thermal technologies: these technologies are able to achieve microbiological safety, without negatively impacting on the quality parameters of the treated product. Examples of non-thermal preservation technologies (NTPT) that could be applied to meat and meat products to inactive or reduce the counts of *Pseudomonas* spp. are high pressure processing (HPP), pulsed electric fields (PEF), cold plasma, ultrasounds, ozone treatment, and irradiation of food (where authorized) (Kordowska-Wiater & Stasiak, n.d; Gertzou et al., 2016; Balamurugan et al., 2018; Gómez et al., 2019; Al-Hilphy et al., 2020; Khanashyam et al., 2022; Rosario et al., 2021). Despite several studies demonstrating the potential use of NTPT as preservation technologies, the real application at industrial scale is still scarce, due to high investment costs, consumers' skepticism, and higher costs of the final product, when compared to the conventional ones.

3.1.4. Biopreservation

Other than these preservation techniques based on novel technologies, one emerging method to extend product's quality and shelf life is based on the application of bioprotective cultures. This new approach is called "biopreservation" and refers to techniques based on the use of living microorganisms, or primary and/or secondary metabolites produced by microorganisms such as organic acids and bacteriocins. The most suitable microorganisms for this application are mainly represented by the lactic acid bacteria.

3.1.5. Hurdles technology approach

Beyond being effective when applied alone, the abovementioned preservation strategies can be applied in combination, following the hurdles technology approach, which is an integrated approach based on the combination of different preservation measures that, acting as hurdles, create hostile condition for microorganisms, resulting in the lack of microbial growth (Khan, Tango, Miskeen, Lee, & Oh, 2017). Hurdles that can be applied in order to control microbial growth are represented by intrinsic factors (e.g., pH, a_w, Eh, and nutrients), extrinsic factors (e.g., storage temperature and atmosphere gas composition) and processing factors (heating, drying, and fermentation) (Hamad, 2012). The synergistic proper application of intrinsic, extrinsic, and processing factors improves microbiological safety, stability, and perceived freshness of the product, while minimizing quality loss.

4. Lactic acid bacteria: A wide heterogeneous group of food-grade microorganisms with antimicrobial properties

Lactic acid bacteria represent a wide group of non-spore-forming, non-respiring Gram-positive, peroxidase positive, catalase and oxidase negative rods and cocci characterized by a fermentative metabolism based on the conversion of sugars to ATP, lactic acid, and other metabolites (Françoise, 2010; Kameník and Dušková, 2016).

The fermentative pathway usually takes place under anaerobic

conditions, but lactic acid bacteria can also grow in oxygen's presence, as they are aerotolerant anaerobes (Stieglmeier et al., 2009). The fermentation carried out by lactic acid bacteria can follow two different pathways: the glycolysis (Embden-Meyerhof pathway) and the 6-phosphogluconate/phosphoketolase pathway (6-PG/PK pathway). Glycolysis mainly results in the production of lactic acid and the metabolism is also referred to as homolactic fermentation. The 6-PG/PK pathway, other than in lactic acid, results in other end products, such as CO_2, acetate, ethanol, and the metabolism is also referred to as heterolactic fermentation. Lactic acid bacteria can grow in various habitats and are usually found in environments rich in nutrients (fermentable carbohydrates, amino acids, salts, fatty acids, and vitamins), such as the mucous membrane of the mouth, vagina of mammals, gastrointestinal tract of humans and animals, and a wide range of food products like meat and meat products, milk and dairy products, and vegetables (Whittenbury, 1964; Toomey et al., 2010).

For lactic acid bacteria, meat and meat products represent a favorable growth habitat thanks to various intrinsic factors such as pH (5–6.5), high a_w (0.95–0.99), availability of nutrients, and extrinsic factors like relative humidity, temperature, and packing atmosphere composition. In this environment, lactic acid bacteria can easily grow acting as spoilage microorganisms, as pro-technological microorganisms, or as bioprotective microorganisms. The lactic acid bacteria most involved in meat spoilage are represented by heterofermentative lactobacilli (as *Latilactobacillus curvatus* and *Latilactobacillus sakei*), heterofermentative leuconostocs, *Carnobacterium* spp. and, on a lesser extent, by homofermentative *Lactobacillus* spp. and *Pediococcus* spp. (Iulietto et al., 2015). As a result of their metabolism, heterofermentative lactic acid bacteria produce significant amounts of undesired catabolites, such as CO_2, ethanol, acetic acid, butanoic acid, and acetoin, thus causing the development of off-odors, package "blowing", and ropy slime on the surface of fresh meat and meat products. Homofermentative lactic acid bacteria produce almost exclusively lactic acid, which has a milder impact on product's spoilage. Other than as spoilage agents, lactic acid bacteria can act in meat and meat products as starer cultures, dominating the process for the development of fermented meat products, such as fermented sausages. In meat and meat products, lactic acid bacteria can also act as bioprotective microorganisms, therefore inhibiting the growth of spoilage microorganisms, thanks to the lowering of meat's pH, the production of organic acids, the competition for nutrients, the ability of forming biofilm, and the production of bacteriocins.

5. Antimicrobial activities of lactic acid bacteria

Lactic acid bacteria have a long history of use in food products, acting as starter cultures to promote and dominate the fermentation process of many fermented foods. Their natural presence in raw material and naturally fermented foods, and the absence of adverse effects to human health gave them GRAS (Generally Recognized as Safe) and QPS (Qualified Presumption of Safety) status, being recognized as safe for human consumption by the FDA (Food and Drug Administration) and the EFSA (European Food Safety Authority). Along years, lactic acid bacteria addition to foods, when referring to probiotic cultures, has also been linked to health benefits, thus creating a good reputation at consumer's level. The main application of lactic acid bacteria to meat and meat products is still represented by their use as starter cultures for fermented sausages, but in the last decades the growing interest in high quality food and the adversity to chemical preservation techniques, prompted meat industry and academic institutions to evaluate their application as natural biopreservatives.

The antimicrobial activity of lactic acid bacteria in meat and meat products is related to five main abilities: i) lowering of pH values, ii) production of organic acids, iii) production of metabolites, iv) nutrients competition with the natural microflora, and v) production of bacteriocins. The decrease in pH values constitutes the main mechanism of biopreservation in fermented meat products such as fermented sausages, thus ensuring microbiological safety (Castellano et al., 2017). Indeed, as a result of their metabolism, lactic acid bacteria produce organic acids such as lactic acid, propionic acid, malic acid, succinic acid, formic acid, and citric acid. Organic acids are able to inhibit the growth of Gram-negative and Gram-positive bacteria, other than unwanted yeasts and molds. The antimicrobial property is exhibited by two main mechanisms, namely cytoplasmatic acidification and accumulation of the dissociated acid anions to a toxic level (Mani-López et al., 2012).

Other than for the production of organic acids, the antimicrobial activity of lactic acid bacteria is also related to the production of biologically active metabolites, such as diacetyl, acetoin, reuterin, reutericyclin, acetaldehyde, and hydrogen peroxide (Egan et al., 2016; Isa & Razavi, 2018; Ibrahim et al., 2021, Barcenilla et al., 2022). In more detail, diacetyl acts by deactivating target microorganism's key enzymes, resulting in a modification of catalytic activity (Nakajima et al., 2003; Hertzberger et al., 2014; Ibrahim et al., 2021). Hydrogen peroxide is synthesized by lactic acid bacteria under aerobic conditions and can destroy cellular components in Gram-negative bacteria trough the oxidation of the SH group of membrane proteins (Ibrahim et al., 2021). Reuterin is a molecule with antimicrobial activity, synthesized by *Limosilactobacillus reuteri*, able to inhibit several Gram-positive and Gram-negative bacteria by inactivating key enzymes such as ribonucleotide reductase (Asare et al., 2020, Ibrahim et al., 2021). Asare et al. (2020) also demonstrated the strong antimicrobial activity of reuterin against *Campylobacter* spp. isolated from poultry meat. Lactic acid bacteria are also able to inhibit the growth of harmful and spoilage microorganisms through a competitive exclusion mechanism, which is based on competition for nutrients, such as carbon, nitrogen, iron, phosphorus, sulfur, hydrogen, calcium, and other metals (Kehl-Fie & Skaar, 2010; Barber & Elde, 2015; Ghoul & Mitri, 2016), and on competition for binding sites (Ibrahim et al., 2021). The last known mechanism in which lactic acid bacteria can exhibit their antimicrobial ability is through the production of bacteriocins. Bacteriocins are ribosomically synthesized peptides able to prevent the growth of Gram-negative and Gram-positive bacteria producing a bactericidal and/or bacteriostatic effect usually targeting bacteria cytoplasmatic membrane. The antimicrobial activity also targets lactic acid bacteria, however, the bacteriocin-producing lactic acid bacteria are immune to their own bacteriocin due to a specific protective immune mechanism (And & Hoover, 2003; Cotter et al., 2013). Bacteriocins produced by lactic acid bacteria have a molecular weight of 3–10 kDa, are electrically neutral, and possess hydrophilic and hydrophobic regions (Eijsink et al., n.d; da Costa et al., 2019). There are still controversies regarding the classification of bacteriocins as they were initially classified into 4 classes (Rea et al., 2011), with the fourth class being represented by large complexes with lipid or carbohydrate portions. However, the fourth class has now been dismissed and identified as bacteriolysins (Güllüce et al., 2013; Liu et al., 2014).

6. Methodology

The present literature review was carried out focusing on the words 'meat spoilage', '*Pseudomonas* in meat products', 'lactic acid bacteria in meat products', 'antimicrobial activity of lactic acid bacteria', 'lactic acid bacteria against *Pseudomonas*', 'natural preservatives in meat'. A search in the Scopus database (www.scopus.com) was conducted in April 2024 spanning from January 2010 to April 2024. In addition, the literature search was performed through the ScienceDirect database (https://www.sciencedirect.com) and the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) in the same time span. The search yielded >1000 hits from the two databases and it was restricted to the papers focusing on the application lactic acid bacteria antimicrobial activity against *Pseudomonas* in meat and meat products, resulting in 12 studies, which are summarized in Table 1 and discussed in the following chapter.

Table 1
Case studies on antimicrobial activity of lactic acid bacteria against *Pseudomonas* in meat and meat products.

Target microorganism	**Biopreservative** microorganism **or** metabolite	**Application form**	**Tested meat product**	**Results**	**Reference**
Pseudomonas fluorescens CGMCC 1.55	Pentocin 31-1 produced by *Lactiplantibacillus pentosus* 31-1	Direct addition to the tested product	Tray-packed chilled pork	Within the first 6 days, the addition of pentocin determined a decrease in *Pseudomonas fluorescens* counts. During the storage time a slight increase was observed, but still lower than the control sample	Zhang et al. (2010)
Pseudomonas spp.	*Latilactobacillus sakei* suspension (7.5 log CFU/g) and *Latilactobacillus curvatus* suspension (7.5 log CFU/g)	Dipping of the samples in the lactic acid bacteria suspension	Vacuum packed beef slices	During the first 3 weeks of storage there was no significant difference between the control and the dipped samples. A significant reduction in *Pseudomonas* spp. counts was found after 28 days of storage	Zhang et al. (2018)
Pseudomonas aeruginosa MTCC 1934	Biosurfactants produced by *Latilactobacillus paracasei* subsp. *tolerans* N2 and *Latilactobacillus casei* subsp. *casei* TM1B	Direct addition to the tested product	Raw ground goat meat	Biosurfactants were able to inhibit the proliferation of *Pseudomonas aeruginosa* MTCC 1934, inducing a 5 log CFU/g reduction of its count. Biosurfactants addition also inhibited lipid oxidation and volatile nitrogen production, extending ground goat meat shelf life to 15 days	Mouafo et al. (2020)
Pseudomonas fragi (on sterile meat) and *Pseudomonas* spp. (on raw meat)	*Latilactobacillus sakei, Latilactobacillus sakei* CECT 4808, *Lacticaseibacillus rhamnosus,* and *Latilactobacillus* spp.	Addition in concentration of 8 log CFU/cm^2 at 8 °C	Irradiated poultry meat and raw poultry meat	In irradiated meat, the difference between control samples and treated samples was less than 0.5 log, while in raw poultry meat a major biopreservation potential was expressed by both *Latilactobacillus sakei* and *Lacticaseibacillus rhamnosus*	Morales et al. (2020)
Pseudomonas spp.	Bacteriocin-producing *Lactiplantibacillus plantarum* LPL-1 and *Staphylococcus xylosus*	Addition in concentration of 7 log CFU/g	Low-salt (30 % NaCl reduction) fermented pork sausage	In comparison to the standard starter cultures (*Pediococcus pentosaceus* and *Staphylococcus xylosus*), the addition of the bacteriocin-producing strain induced a reduction of *Pseudomonas* spp. counts of 3 log CFU/g in 11 days of ripening instead of 31 days	Zhang et al. (2020)
Pseudomonas spp.	Protective cultures obtained with different combinations of *Latilactobacillus sakei, Pedioccoccus pentosacesus*, *Staphylococcus xylosus* and *Staphylococcus carnosus*	Direct addition of protective cultures to the tested product	MAP and vacuum packed (VP) lamb meat	In MAP-stored meat samples, no statistically significant reduction in *Pseudomonas* counts was observed, while a reduction of 0.9 and 1.2 log CFU/cm^2 was observed in vacuum-packed treated samples containing *Latilactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus,* or *Staphylococcus carnosus* plus *Latilactobacillus sakei*	Xu et al. (2021)
Pseudomonas spp.	Two protective culture preparations: i) *Latilactobacillus sakei* (L); ii) *Staphylococcus carnosus* plus *Latilactobacillus sakei* (SL)	Direct addition in concentration of 7.5 and 7.7 log CFU/mL, respectively for (L) and (SL)	Vacuum packed (VP) lamb meat	The addition of protective cultures showed a significant inhibitory effect against the growth of *Pseudomonas*, resulting in final counts after 20 days of storage of 3.6 log CFU/cm^2 (L) and 3.3 log CFU/cm^2 (SL). The final counts in untreated samples reached 5.2 log CFU/cm^2 at day 20	Xu et al. (2022)
Pseudomonas spp.	*Lactiplantibacillus paraplantarum* BPF2 and *Pediococcus acidilactici* ST6	Direct addition in concentration of 6.3 log CFU/g	Traditional spanish fermented sausage (*salchicón*)	Both *Lactiplantibacillus paraplantarum* BPF2 and *Pediococcus acidilactici* ST6 were able to induce a reduction in Pseudomononadaceae counts. Metagenomic analysis showed a reduction of Pseudomonadaceae from 42.7 % to 0.9 % (treatment with *Lactiplantibacillus paraplantarum* BPF2) and 2.2 % (treatment with *Pediococcus acidilactici* ST6) of the relative frequency	García-López et al. (2023)
Pseudomonas spp.	Lyophilized/freeze-dried paraprobiotic (LP) alone (3 %; 6 %) or in combination with EDTA (3 % LP + EDTA; 6 % PL + EDTA)	Direct addition to the tested product	Beef meatballs	All treatments were able to retain constant or decrease *Pseudomonas* spp. counts. The most effective treatment, in terms of antimicrobial activity, was the 6 % LP + EDTA, which resulted in a reduction of 4 log cycles	Kürşad Incili et al. (2023)
Pseudomonas spp.	*Latilactobacillus sakei* RS-25	Direct addition in concentration of 6 log CFU/g	Overwrapped beef steak	No significant difference in *Pseudomonas* counts was found in the inoculated samples during the 12 days of 4 °C chilled storage. *Latilactobacillus sakei* RS-25 had no inhibitory effect on *Pseudomonas*	Yang et al. (2023)
Pseudomonas fluorescens	Pool of *Carnobacterium maltaromaticum* strains (CM_B824, CM_B827, CM_B289)	Direct addition in concentration of 6.4 log CFU/mL	Ground beef and sliced cooked ham	The pool of *Carnobacterium malatormaticum* strains was able to reduce the counts of both indigenous and inoculated *Pseudomonas fluorescens*, resulting in a potential increase of both	de Andrade Cavalari et al. (2024)

(*continued on next page*)

Table 1 (*continued*)

Target microorganism	Biopreservative microorganism or metabolite	Application form	Tested meat product	Results	Reference
				ground beef and sliced cooked ham's shelf life	
Pseudomonas fragi	*Latilactobacillus sakei*, *Latilactobacillus sakei* CECT 4808, *Lacticaseibacillus rhamnosus* and *Latilactobacillus* spp. used as broth coltures (TSBYE)	In vitro addition in concentration of 8 log CFU/mL at 8 °C	In vitro testing	*Latilactobacillus sakei* and *Latilactobacillus sakei* CECT 4808 exhibited the greatest biopreservation potential against *Pseudomonas fragi*, resulting in 99.99999 % inhibition rate, while *Lacticaseibacillus rhamnosus* inhibition rate was 99.9 %	Morales et al. (2020)
Pseudomonas aeruginosa	Supernatants obtained from 8 strains of *Lactiplantibacillus plantarum*, 3 strains of *Ligilactobacillus salivarius*, 2 strains of *Pediococcus pentosaceus*, 1 strain of *Lactobacillus acidophilus*, 1 strain of *Lactobacillus gallinarum,* 1 strain of *Lactobacillus gasseri,* and 1 strain of *Limosilactobacillus fermentum*	In vitro addition of the supernatants obtained from 17 lactic acid bacteria strains	In vitro testing	Among the 17 lactic acid bacteria strains, *Lactipantibacillus plantarum* LPyang strain showed the ability to inhibit the growth, pyocyanin expression, and biofilm formation of *Pseudomonas aeruginosa*	Li et al. (2023)

7. Application of lactic acid bacteria against *Pseudomonas* spp. in meat and meat products

During the past decade, the antimicrobial potential of lactic acid bacteria against *Pseudomonas* spp. has been investigated, as shown in Table 1. The reported studies investigated the antimicrobial activity of different lactic acid bacteria cultures and their metabolites used directly in meat and meat products or after *in-vitro* experiments, highlighting the potential application of selected lactic acid bacteria strains as natural preservatives against *Pseudomonas* spp. in meat and meat products.

Fig. 1 summarizes the practical application of lactic acid bacteria cultures or metabolites (bacteriocins) to meat and meat-based products.

7.1. Direct addition of lactic acid bacteria in raw meat

In 2010, Zhang and colleagues evaluated the potential bioprotective effect against *P. fluorescens* strain CGMC 1.55 of a lactic acid bacteria bacteriocin (pentocin 31-1 produced by *Lactiplantibacillus pentosus* 31-1 isolated from traditional China fermented Xuan-Wei Ham) added to non-vacuum tray-packed chilled pork. In the study by Zhang et al. (2010), pentocin was obtained through batch fermentation with controlled pH and temperature. Four different preservative solutions were prepared: 75 AU/mL (Activity Unit per milliliter) nisin, 40 AU/mL pentocin 31-1, 80 AU/mL pentocin 31-1, and sterilized distilled water (used as control). Meat samples were initially inoculated with *P. fluorescens* CGMCC 1.55 (5×10^5 CFU/g) immersed in the four different preservative solutions for 15 s and dripped for 3–4 min. After dripping, meat samples were packed in sterile plastic trays and stored at 4 °C. The results of the study showed the potential ability of 80 AU/mL pentocin 31-1 to extend chilled pork meat shelf life up to 15 days, whereas the samples treated with 70 AU/mL nisin and 40 AU/mL pentocin 31-1 could only reach 12 days. Compared to control sample, *P. flurescens* GCMCC 1.55 counts

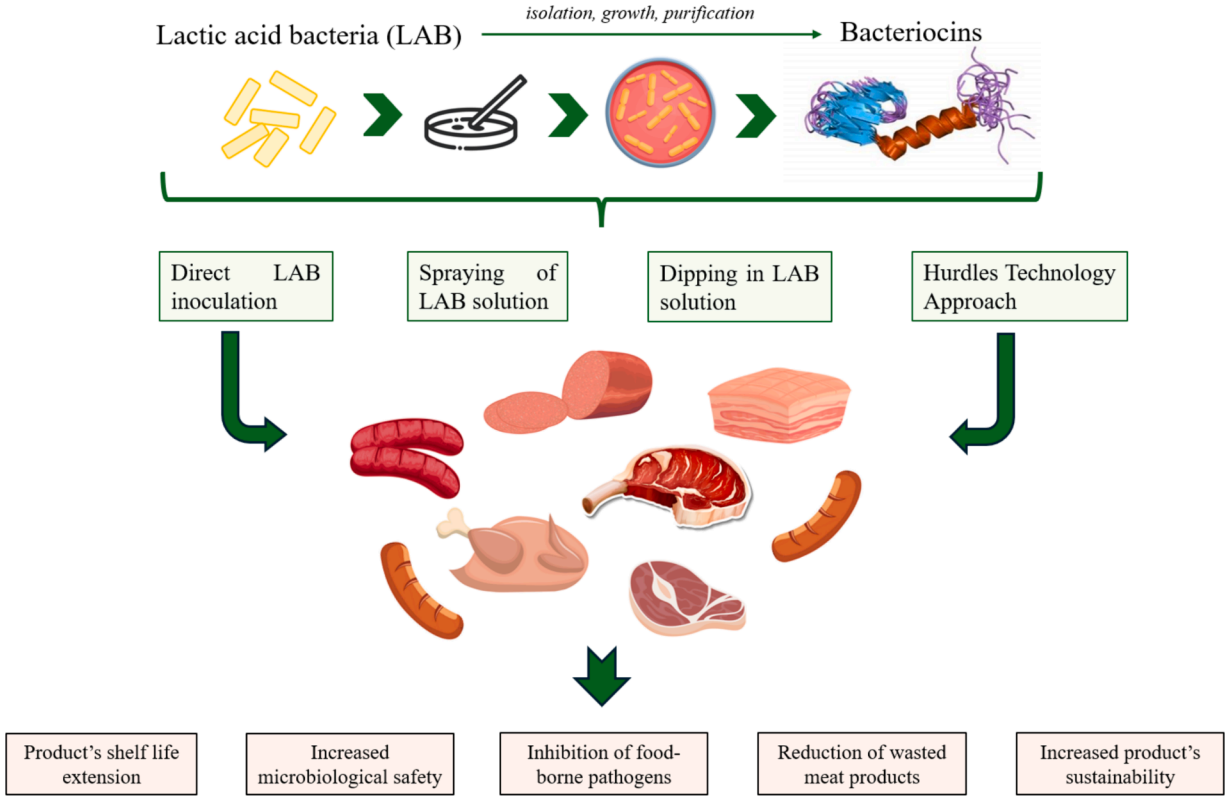

Fig. 1. Application methods of lactic acid bacteria cultures or metabolites (bacteriocins) to meat and meat-based products.

were 2 log CFU/g lower in samples treated with 80 AU/mL pentocin 31-1. Therefore, pentocin 31-1 showed the ability to extend products shelf life, thus inhibiting *P. fluorescens* while retaining good sensory characteristics.

Mouafo et al. (2020) used biosurfactants produced by *Lacticaseibacillus paracasei* subsp. *tolerans* N2 and *Lacticaseibacillus casei* subsp. *casei* TM1B as biopreservatives against *Pseudomonas aeruginosa* MTCC 1934 and *E. coli* MTCC 118 in raw ground goat meat stored at 4 °C for 15 days. The authors used two solutions of the biosurfactants in sterile distilled water at two different concentrations (0.02 mg/mL and 0.05 mg/mL). Goat meat samples were inoculated by immersion for 1 h in 1 L of a bacteria cocktail (composed of 500 mL of 7 log CFU/mL of *P. aeruginosa* MTCC 1934 and 500 mL of 7 log CFU/mL of *E.* coli MTCC 118) at 4 °C. After draining and incubation for 12 h, meat samples, in batches of 500 g, were mixed for 5 min in sterile polyethylene bags with 100 mL of biosurfactant solution and grounded twice with a 3.2 mm plate. After grinding, meat samples were wrapped in sterile polyethylene bags and stored at 4 °C. In addition to the negative control (NC) and to the 4 inoculated samples (N2, N5, T2, T5), a positive control (PC) was obtained by collecting a sample of ground goat meat prior to the addition of biosurfactants. The results showed that when meat samples were treated with the antimicrobial compound, a significant reduction ($P > 0.05$) in *P. aeruginosa* MTCC 1934 counts was recorded when compared to the positive control. This reduction demonstrated the ability of biosurfactants produced by *L. paracasei* N2 and *L. casei* TM1B to inhibit the proliferation of *P. aeruginosa* and other spoilage microorganism, thus extending raw ground got meat shelf life up to 15 days at 4 °C. The authors stated that the antimicrobial activity of biosurfactants produced by lactic acid bacteria against *P. aeruginosa* MTCC 1934 might be due to their ability to alter the cell wall and the outer membrane of the bacteria, as also reported by Hippolyte et al. (2018).

Morales et al. (2020) compared 3 different antagonistic assays using 4 lactic acid bacteria (*L. sakei*, *L. sakei* CECT 4808, *Latilactobacillus* spp., and *Lacticaseibacillus rhamnosus*) *in situ* on irradiated sterile chicken meat and on raw chicken meat. Lactic acid bacteria were inoculated (8 log CFU/mL) alone in raw chicken meat and in combination with *P. fragi* (3 log CFU/mL or cm^2) in sterile chicken meat. *P. fragi* was previously isolated from skinless marinated poultry breast fillets and was used as indicator to evaluate the antimicrobial potential of the 4 tested lactic acid bacteria. *In situ* testing in irradiated chicken meat showed that only *L. sakei* was able to exhibit a slight antagonistic effect against *P. fragi*, thus lowering its counts of 0.5 log CFU/cm^2. According to the authors, this could have occurred because *P. fragi* was isolated from poultry meat, so it was supposed that the microorganisms was particularly well adapted to grow in that specific environment. Moreover, the irradiation process removed any another microbial competition except for the inoculated lactic acid bacteria strains. *In situ* testing in fresh chicken meat evidenced that both the tested *L. sakei* strains had a marked biopreservative potential against *Pseudomonas* spp., with some minor antagonistic effect from *Latilactobacillus* spp. Both the *L. sakei* strains during 4 days of storage at 8 °C produced an acid smell and a superficial slime, but the samples were not rejected by a panel of 5 untrained panelist. The authors stated that the greater antimicrobial activity exerted against *P. fragi* in raw chicken meat rather than irradiated chicken meat may be related to the synergic effect of lactic acid bacteria and concomitant microbiota present in fresh chicken meat.

Xu et al. (2021) evaluated the antimicrobial potential against *Pseudomonas* spp. of six different protective cultures (obtained by different combinations of *L. sakei*, *Pediococcus pentosaceus*, *Staphylococcus xylosus,* and *Staphylococcus carnosus*) on chilled lamb meat packed under different packaging systems (MAP and vacuum packaging) and stored at 4 °C. Fresh lamb backstraps (*longissimus lumborum*) were dipped in the protective cultures for 30 s and then aseptically drained for 5 min in a laminar flow cabinet. A total of 8 test groups were obtained: untreated control sample, sterile water-dipped sample, group C1 (*L. sakei*), group C2 (*P. pentosaceus* and *S. xylosus*), group C3 (*L. sakei*, *S. carnosus*, *S. xylosus*), group C4 (*S. carnosus*, *L. sakei*), group C5 (*S. carnosus* and *P. pentosaceus*) and group C6 (*P. pentosaceus* and *S. xylosus*). Each group was tested under two different packaging systems: MAP with a gas mixture of 80 % oxygen and 20 % carbon dioxide (stored for 7 days at 4 °C) and vacuum packaging (VP) at a final vacuum of 99 % (stored for 15 days at 4 °C). Initial numbers of *Pseudomonas* spp. were 3.0 log CFU/cm^2 for both MAP- and VP-stored samples. In untreated samples stored under MAP, *Pseudomonas* counts increased to 4.4 log CFU/cm^2, whereas in culture-treated samples its numbers were 0.8 log CFU/cm^2 lower (except for C3 samples, in which *Pseudomonas* counts were 0.2 log CFU/cm^2 higher than the control). These differences were reported to be statistically insignificant by the authors. In VP stored samples, *Pseudomonas* counts reached numbers of 4.8 log CFU/cm^2 in untreated sample, whereas a statistically significant reduction of 0.9 log CFU/cm^2 and 1.2 log CFU/cm^2 was observed in C3 and C4 samples, respectively. The antimicrobial effect of the other tested treatments was slight or absent. According to the authors, these results showed that the C3 and C4 treatments could have contributed to a spoilage delay and shelf-life extension in chilled lamb meat when combined with vacuum packaging, by inhibiting *Pseudomonas* and other bacteria.

The same authors, in 2022, applied two protective cultures (one containing *L. sakei* – L – and the other containing *S. carnosus* and *L. sakei* – SL) to vacuum-packed lamb meat stored at chilling temperature (4 °C). The concentration of starter culture suspensions was 7.5 log CFU/mL for culture L and 7.7 CFU/mL for culture SL. Lamb meat samples were treated by dipping in the protective culture suspension for 30 s. Later, meat samples were left to dry for 5 min and then vacuum-packed and stored at 4 °C for 20 days. During the storage period, the two protective cultures were evaluated for the antimicrobial potential against *Pseudomonas* spp. growth. Initial *Pseudomonas* spp. counts were 2.6 log CFU/cm^2 in all samples and the growth was observed from day 10 of storage. Both protective cultures were able to inhibit *Pseudomonas* spp. growth, resulting in final counts of 3.6 and 3.3 log CFU/cm^2 for L and SL cultures, respectively. *Pseudomonas* population reached numbers of 5.2 log CFU/cm^2 in the untreated samples, therefore both protective cultures exhibited an inhibitory effect against *Pseudomonas* spp., contributing to an overall spoilage delay and shelf-life extension of the product (Xu et al., 2022).

7.2. Direct addition of lactic acid bacteria in meat products

Zhang et al. (2020) investigated the impact of the addition of bacteriocin-producing *Lactiplantibacillus plantarum* LPL-1 on microbial dynamics in low-salt fermented sausages (30 % salt reduction of normal content of 3 %). *L. plantarum* LPL-1 was inoculated with *S. xylosus* at 7 log CFU/g. The addition of this bacterial cocktail induced a marked reduction in *Pseudomonas* spp. counts, resulting in less than 1 log CFU/g after 11 days of ripening. The same result was reached after 21 days of ripening in the control sample obtained through the addition of a standard starter mixture (*P. pentosaceus* with *S. xylosus*). Therefore, the authors stated that *L. plantarum* LPL-1 addition in low-salt fermented sausages inhibited the growth of spoilage bacteria, lowering product's pH and showing a great potential to be used as starter culture to improve quality and safety of low-salt fermented sausages.

García-López et al. (2023) tested the antimicrobial effect of two autochthonous lactic acid bacteria strains (*Lactiplantibacillus paraplantarum* BPF2 and *Pediococcus acidilactici* ST6) previously isolated from spontaneously fermented sausages produced in Spain (*salchicón*). Starter cultures were added to a final concentration of 6.3 log CFU/g and the ripening process was carried out at 18 °C for 10 h, then, samples were maintained a 24 °C for 48 h, and the products were dried for 28 days in a chamber with 80 % humidity and a temperature of 14 °C. The addition of the two lactic acid bacteria strains was evaluated in comparison with the initial meat batter (prior to starter culture addition), with a spontaneously fermented sample, and with sausages obtained through the addition of a commercial starter culture (RAP). For each

sample, a metagenomic analysis was carried out to study the microbial population involved in the process. The analysis performed in the initial meat batter highlighted *Pseudomonas* as the most relevant taxon (42.7 %). In the end product, *Pseudomonas* presence was strongly reduced in spontaneously fermented sausages, representing 3.5 % of total population, and it was also strongly inhibited by the addition of the commercial starter culture (RAP) (*Pseudomonas* represented 1.0 % of total population). A reduction in *Pseudomonas* prevalence (%) was obtained also through the addition of the two autochthonous tested lactic acid bacteria, resulting in a reduction of *Pseudomonas* presence to 0.9 % (*L. paraplantarum* BFP2) and to 2.2 % (*P. acidilactici* ST6).

Yang et al. (2023) assessed the inhibitory effect of *L. sakei* RS-25 on the spoilage of overwrapped beef steaks stored at 4 °C for 12 days. *L. sakei* RS-25 was previously isolated from chilled beef and was then used to contaminate beef samples. In the tested group, *L. sakei* RS-25 was sprayed at 6 log CFU/g, whereas the control samples were obtained through spraying of sterile water. After the spaying process, samples were let to dry, transferred to tray packs, and wrapped with PE film. Beef samples were stored at 4 °C for 12 days and analyzed. *Pseudomonas* spp. counts showed that lactic acid bacteria treatment was not able to reduce the counts of the target microorganisms, resulting in no significant differences during the 12 days of storage. Therefore, the authors stated that *L. sakei* RS-25 had no inhibitory effect on *Pseudomonas* during the storage period of the tested beef steaks, whereas it was able to inhibit the growth of *Salmonella* Thyphimurium and *B. thermosphacta* whitin 6 days of storage. Of note, this evidence likely suggests a genus- or species-specific action of the tested lactic acid bacteria.

In 2024, de Andrade Cavalari et al. evaluated the bioprotective effect of *Carnobacterium maltaromaticum* against *P. fluorescens* in ground beef (stored for 3 days at 4 °C and 4 days at 8 °C) and sliced cooked ham (stored for 10 days at 4 °C and for 18 days at 8 °C). The authors evaluated the effect of the addition of a pool of three *C. maltaromaticum* strains (CM_B824, CM_B827, CM_B289) previously isolated from vacuum-packed Australian bovine *longissimus thoracis et lumborum*. Ground beef was previously inoculated with *C. maltaromaticum* (CM) at a concentration of 6.8 log CFU/mL, mixed for 2 min, then, beef was inoculated with *P. fluorescens* at a concentration of 3.5 log CFU/mL and mixed for 2 min. After contamination, beef patties were formed, packed in PP/EVOH/PP trays, filled with MAP containing 66 % O_2, 4 % N_2 and 30 % CO_2, sealed with PET/PP film, and stored for 7 days. The addition of *C. maltaromaticum* resulted in a significant inhibition of the growth of both inoculated and autochthonous *P. fluorescens*. In sliced cooked ham, the inoculation was performed through the immersion of the slices in 1000 mL of saline solution (0.9 %) containing the bacterial inoculum: 6.4 log CFU/mL of *C. maltaromaticum* and 3.8 log CFU/g of *P. fluorescens*. The slices were maintained in contact with the suspension for 10 min and the left to dry for 10 min. After drying the samples were packed in PP/EVOH/PP trays, filled with MAP containing 70 % N_2 and 30 % CO_2, sealed with PET/PP film and stored for 28 days. As previously described for ground beef, *C. maltaromaticum* was able to inhibit *P. fluorescens* growth also in sliced cooked ham, resulting in a significant reduction in its counts. According to the authors, the inhibitory effect of *C. maltaromaticum* might be related to: competition for nutrients; production of antagonist compounds (diacetyl, CO_2); faster growth and bacteriocin production in the matrix; production of organic acids (lactic, formic, and acetic acid) (de Andrade Cavalari et al., 2024).

In 2023 Kürşad Incili et al. evaluated the anti-*Pseudomonas* potential of lyophilized/freeze dried paraprobiotic (LP) containing *P. acidilactici* B-LC-20 in ground beef meatballs. The meatballs were divided in 6 groups: control group (no addition), EDTA (0.02 M) group, 3 % paraprobiotic (LP) group, 3 % paraprobiotic (LP) plus EDTA (0.02 M) group, 6 % paraprobiotic (LP) group, and 6 % paraprobiotic (LP) plus EDTA (0.02 M) group. The manufactured samples were packed in sterile trays and stored under aerobic condition at 4 °C for 8 days. In the control sample, *Pseudomonas* spp. counts gradually raised during storage period, whereas its counts were lower in the treated samples. The higher antimicrobial activity was provided by the combination of 6 % LP and 0.02 M EDTA in which *Pseudomonas* spp. counts were 4.47 log CFU/g lower than the control sample on the 8th day of storage (Kürşad Incili et al., 2023).

7.3. Dipping in lactic acid bacteria suspensions

Zhang et al. (2018) evaluated the bioprotective potential of *L. sakei* (Bactoferm B-2, supplied by Chr. Hansen) and *L. curvatus* (SafePro B-LC-48, supplied by Chr. Hansen) in vacuum-packed chilled beef steaks. Differently from the study by Zhang et al. (2010), the authors tested the direct addition of lactic acid bacteria, instead of their bacteriocins. In more detail, beef steaks were dipped into two potentially bioprotective suspensions (7.5 log CFU/g of *L. sakei* and 7.5 log CFU/g of *L. curvatus*, respectively) for 30 s, and then drained aseptically for 1 min. Steaks without any treatment were used as control samples. PCR-DGGE profile analyses showed that, in control samples, *P. fragi* emerged during the early days of storage, whereas *P. putida* multiplied during the mid-storage time and became the predominant bacteria. With the inoculation of both *L. sakei* and *L. curvatus*, *P. fragi* was completely inhibited during the 38 days of storage, whereas *P. putida* was temporarily inhibited and only multiplied during the late storage days. Viable counts confirmed the antimicrobial potential of both strains, with *L. sakei* exerting a strong inhibitory effect against *Pseudomonas* spp. after day 28 of storage, thus producing a significant reduction in *Pseudomonas* spp. counts.

7.4. In vitro testing of lactic acid bacteria

In the same study mentioned in paragraph 7.1, Morales et al. (2020) also compared the 3 different antagonistic assays using 4 lactic acid bacteria (*L. sakei*, *L. sakei* CECT 4808, *Latilactobacillus* spp., and *L. rhamnosus*) *in vitro*, on broth culture (TBSYE growth medium). Lactic acid bacteria were inoculated (8 log CFU/mL) in combination with *P. fragi* (4 log CFU/mL) in TBSYE (pH 5.7–5.9) at 8 °C. In vitro testing showed that both the *L. sakei* tested strains had the greatest antimicrobial potential against *P. fragi* in TBSYE at 8 °C, with an inhibition rate of 99.99999 %, whereas *L. rhamnosus* showed an inhibition rate of 99.99 %. The authors stated that the bioprotective activity was mainly related to *L. sakei* ability to produce organic acids and reduce pH values of the medium (reaching a pH of 4.4 in 4 days). *Latilactobacillus* spp. reached pH values of 5.0 in 4 days and resulted in a reduced antimicrobial activity.

7.5. In vitro testing of lactic acid bacteria supernatants

Li et al. (2023) tested the *in vitro* antimicrobial potential of 17 strains of lactic acid bacteria against *P. aeruginosa*. The target microorganism (*P. aeruginosa*) was cultured on LB agar plates with a 6 mm disc placed into the plate. Then, 150 μL of the different lactic acid bacteria cultures were added to the discs, in order to evaluate their antimicrobial potential against *P. aeruginosa*. The antimicrobial potential was determined by measuring the diameter of the inhibition zones surrounding the disc after 18–24 h of incubation at 37 °C. The results showed that six lactic acid bacteria strains (H5, H9, H11, H12, H14, and H17) determined a significant antimicrobial effect against *P. aeruginosa*, with an inhibition zone of about 20 mm. Among them, the H17 strain (*L. plantarum*) was also able to determine a high inhibition of *P. aeruginosa* biofilm formation.

8. Conclusion

Lactic acid bacteria have mainly been used along years to guide the production process of fermented meat products, playing a crucial role in the development of product's quality, flavor, and texture.

In the reviewed studies, lactic acid bacteria antimicrobial activity

was tested both *in vitro* and in real meat systems, showing a higher inhibitory potential agains *Pseudomonas* spp. when tested in synthetic growth media rather than in meat. In more detail, the *in vitro* studies were usually used as a preliminary screening activity followed by testing in a real meat product, in which other variables such as the matrix effect and competition for nutrients with the autochthonous microorganisms might have influenced the activity of the tested bioprotective cultures. In the real meat system, different authors evaluated the anti-*Pseudomonas* activity related to the addition of metabolites produced by lactic acid bacteria (e.g., bacteriocins and biosurfactants) and to the direct addition of viable lactic acid bacteria. The results overall collected highlighted a stronger antimicrobial activity exhibited by the addition of bacterial metabolites, inducing a reduction in *Pseudomonas* loads, whereas the reduction related to the addition of viable lactic acid bacteria was lower. Based on this evidence, the difference in *Pseudomonas* inhibition rate may be linked to the fact that when adding bacteriocin-producing lactic acid bacteria, the antimicrobial activity is strictly related to the ability of producing the bacteriocin in the real meat system. Of note, the addition of isolated compounds can bypass this limit, therefore inducing a higher antimicrobial activity against *Pseudomonas* present in the meat environment. Moreover, the addition of isolated microbial compounds could overcome the issue related to the potential spoilage activity exerted by viable lactic acid bacteria deliberately added to meat, although the costs of pure bacteriocins are still to be evaluated and faced.

Interestingly, several studies have evaluated the antimicrobial activity of *L. sakei*, which is known to be well adapted to the meat environment, being one of the dominant species in fermented sausages. *L. sakei* activity was investigated by different authors, in different types of meat, and under different packaging conditions. The stronger antimicrobial effect against *Pseudomonas* spp. was observed when *L. sakei* was added to vacuum packed lamb and beef meat, whereas no significant differences were observed in lamb meat stored under MAP. These results may be related to the fact that *Pseudomonas* spp. is aerobic or facultatively anaerobic. Hence, under anaerobic conditions, *Pseudomonas* finds a hostile environment that allows *L. sakei* to express an enhanced its antimicrobial activity.

Other than the packaging conditions, another important factor that can influence the biopreservative potential of lactic acid bacteria is the level of contamination. In raw meat, the presence of concomitant bacteria can result in a synergic antagonistic effect against *Pseudomonas* growth, due to competition for nutrients and production of antimicrobial compounds such as organic acids. In sterile meat (e.g., irradiated meat or cooked meat), in case of contamination with *Pseudomonas*, the antimicrobial activity of the added lactic acid bacteria strain may result weak due to the absence of other microorganisms competing with *Pseudomonas*. Then, it can be stated that the antimicrobial activity of lactic acid bacteria directly added to meat or meat products is affected by the environmental conditions of the product in which they are inoculated. No significant differences were observed when testing different types of meat (beef, chicken, pork, or lamb). However, the effectiveness of the antimicrobial activity against *Pseudomonas* in non-fermented meat and meat products needs to be further investigated in order to understand the influence of the type of end product, the type of packaging, and the storage conditions (time and temperature). Moreover, the antimicrobial activity of lactic acid bacteria must also be evaluated taking into consideration their ability to maintain the sensory features of fresh meat (whether whole or minced), without negatively affecting its sourness and acidity. The results herein discussed emphasize the challenges occurred in translating *in vitro* findings to practical applications due to the complex interactions between bacteria, antimicrobial metabolites, and food matrices.

CRediT authorship contribution statement

Valerio Marcelli: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Andrea Osimani:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Lucia Aquilanti:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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