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# Lukanka, a Semi-Dried Fermented Traditional Bulgarian Sausage: Role of the Bacterial Cultures in its Technological, Safety and Beneficial Characteristics

**Svetoslav Dimitrov Todorov[1*], Clarizza May Dioso[2], Min-Tze Liong[3], Tonka Vasileva[4] Penka Moncheva[5], Iskra Vitanova Ivanova[5], Ilia Iliev[4]**

1-ProBacLab, Department of Advanced Convergence, Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea.

2-Institute of Biological Science, University of the Philippines Los Baños, Laguna, Philippines.

3-School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia.

4-Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria.

5-Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of General and Industrial Microbiology, Sofia, Bulgaria.

## Abstract

**Background and Objective:** Fermentation, smoking and salting processes of raw materials are parts of the preservation processes that are essential for the production of final products in south European countries. Originally, fermentation of meat products was used for long-term preservation and safe storage. However, gastronomical characteristics of the fermented meat products are now essential in achieving favorable flavor, aroma, color and structure of the salamis that are preferred by the consumers. The emphasis is on the gastronomic characteristics that are resulted from various combinations of raw meats, specific spices and the natural microbiota or fermentation processes carried out using specific starter cultures. The objectives of this review were to provide information regarding origin of *lukanka*, a Bulgarian semi-dried fermented meet product, knowledge about microbiological specificity of the product and examples of beneficial characteristics associated with lactic acid bacterial isolated from *lukanka*.

**Results and Conclusion:** Meat starter cultures typically consist of seven bacterial species belonged to *Lactobacillus*, *Leuconostoc*, *Staphylococcus*, *Enterococcus*, *Lactococcus*, *Micrococcus* and *Streptococcus* Spp. Various lactic acid bacteria were isolated from *lukanka* and assessed regarding their safety and beneficial characteristics. During the maturation of fermented meat products, complex fermentation processes ensue from the interactions between the bacterial starter cultures, residual enzymes in the muscles and fat tissues and available bacterial enzymes. Roles of beneficial characteristics of lactic acid bacterial from *lukanka* were discussed as essential for the designation of *lukanka* as a functional food product. Traditional fermented meat products are specific ecological systems, where interactions between various bacterial strains and food matrices result in formation of the unique characteristics of the final product. Analyzing traditional fermented meat sausages from the perspective of 21 century science point of view, these foods can be addressed as examples of functional food products, providing nutritional, gastronomical and beneficial advantages to the consumers.

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**Corresponding author:**

**Svetoslav Dimitrov Todorov***

ProBacLab, Department of Advanced Convergence, Handong Global University, Pohang, Gyeongbuk 37554, Republic of Korea

Tel: +82-10-34903152
E-mail: slavi310570@abv.bg

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## 1. Introduction

Production of the fermented meat sausages is generally linked to European countries in the Old World. Climate-specific, social-economic identity of the regions and moderated nutritional specificity have resulted in distinct culinary regions across the Old World's social and economic development. Preparations of fermented meats or simply dry

meat products can be traced back to cavemen practices, as this was their natural method of food preservation. Later when animals were domesticated, dairy production transited from nomadic to a more settled lifestyle. Agricultural processes accelerated production and consumption of fermented fruits, vegetables and cereals. From a historical perspective, movement of large groups of people and tribes at various phases of Eurasian history has resulted in interchange of information regarding the distribution of various floral and animal species across the European continent. This has also resulted in the spread of many microbial cultures. During the period of Great Geographical Discoveries, new plants and animals from new territories were transferred to Europe, carrying beneficial and harmful microorganisms with them [1,2]. Eating habits clearly played leading roles in the beginning of human civilization when analyzing development of nutritional habits and their roles in human evolution and civilization. Fermented foods included nutritional requirements and were the only way to preserve food during famines [3]. In modern era, abandoned food resources are not addressed for the development of fermented food products because fresh foods are available all the year. Similarly, regions with extreme weather and limited food resources developed fermented food products as novel ways to preserve foods to ensure sufficient food supplies all the year [1-3]. For the longest time in human history, fermented food products were consumed primarily as nutritional commodities. Gastronomical characteristics were only recognized by consumers in the last few centuries. This also is true for the fermented meat products. The modern society characterizes gastronomical characteristics of various fermented meat products and classifies them as a part of human civilization gastronomical heritage [3].

Specific Mediterranean climate conditions affected the production of fermented foods, especially fermented meats. Refrigeration, sterilization and canning are examples of the last century preservation techniques. Thousands of years ago, the Mediterranean civilization was built on fermentation and biopreservation. Mediterranean fermented foods are nutriational and gastronomic sources [4]. Fermented foods are referred as essential components of these Mediterranean countries' non-material cultural history and admired for their nutritional and gastronomic benefits. These traditional foods are rich sources of microbiological starter cultures for traditional and innovative fermented meat products, according to microbiologists. To preserve traditional characterristics and features of the fermented meat sausages, it is vital to understand not only the technological processes but also their microbial ecology and biodiversity [1,3,4]. In the recent decade, spontaneous fermentation-based fermented foods have become popular. Natural meat fermentation can affect product homogeneity even after boosting indigenous microbiota and selecting new starter cultures. This relies on the autochthonous. Due to these variables, commercial production of the fermented foods depends on well-designed starting cultures, which are suggested for fermented meats. Starter cultures are critical in industrial and homemade productions of the fermented meats [5]. The goal is to improve the quality, decrease inconsistency and improve sensorial properties of the fermented food products [6,7]. Moreover, researchers are investigating novel starter cultures that can produce health-associated metabolites, include beneficial characteristics and lower levels of biogenic amines (BA) and cholesterol in fermented meat products [7]. Furthermore, starter culture safety must be assessed before a novel culture can be approved for commercial uses.

Probiotics are defined as live microbial organisms which, when consumed in appropriate quantities, can generate specific health promoting effects for the host. Majority of the commercialized probiotics are strains belonged to various *Bifidobacterium* and *Lactobacillus* spp. [8]. In recent years, specific strains belonged to *Lactococcus* spp., *Enterococcus* spp., *Saccharomyces* spp. and *Propionibacterium* spp. were extensively assessed as promising probiotics with beneficial characteristics and have been suggested to include health-promoting characteristics [9]. Extensive studies from the last decade have shown beneficial potential of supplementing with probiotic cultures to fermented meat products such as various sausages to deliver the beneficial microorganisms to the consumers. Erkkila et al. [10] reported fermented dry sausages, prepared with three various strains of *Lacticaseibacillus* (*Lb*.) *rhamnosus,* which were previously reported as probiotics. Pennacchia et al. [11] suggested use of *Lb. plantarum* and *Lb. paracasei* in industrial meat products and highlighted their probiotic characteristics. *Pediococcus acidilactici* and *L. sakei* demonstrated decent subsistence characteristics in fermented meat products. These two strains were suggested as decent putative probiotics with beneficial roles in fermented meat products [12]; Todorov et al. [13] assessed safety associated characteristics of *Lb. sakei*, *Lb. plantarum* and *Enterococcus faecium* strains isolated from fermented meat products traditionally produced in Iberic Peninsula for their use as beneficial cultures (starters, bacteriocin producers and probiotics). Bacteriocin-producing starter cultures can be advantageous because they can compete with the potentially contaminate spoilage bacteria, including pathogens, in the food matrix and thus can be well-thought-out as probiotics. In a similar study [14], researchers investigated effects of bacteriocinogenic strains of *Lb. plantarum* and its bacteriocin negative mutant in production of various types of salamis. The study showed that production of antimicrobial peptides could contribute to the safety of the fermented salamis. From a variety of meat-based salamis, researchers have demonstrated that *Listeria monocytogenes* load decreased when fermented with a bacteriocin-producing strain (*Lb. plantarum* 423) [14]. Moreover, sensorial properties of the prepared sausages did not differ when the production process included the non-

bacteriocin producing mutant of *Lb. plantarum* 423, as well as an industrial starter culture (*Lb. curvatus*). This study clearly showed that selection of appropriate starter cultures with bacteriocinogenic characteristics could be useful for the decrease of food spoilage and foodborne pathogens and actively contribute to the safety of the final products. In addition, a few bacteriocinogenic strains have been isolated from various fermented foods and suggested for use as biopreservatives. Authors have suggested that fermented salamis can be considered as vectors for providing of probiotic bacteria to the customers and advocating that traditional salamis produced with beneficial cultures can be classified as functional food products (for more details see Vazquez Bucheli et al. [15]). The aims of the present review were to summarize information on the characterization of the specific microbiota associated to *lukanka*, a naturally-fermented semi-dried Bulgarian sausage and provide arguments in links between the fermented meat products and general wellbeing. *Lukanka* is a well-known Bulgarian semi-dry fermented meat product but relatively low studied. With objective to collect and digest the known information on *lukanka*, the current authors have targeted to accelerate the scientific curiosity and generate deeper research not only on Bulgarian fermented meat products but also on various traditional fermented food products, which were known for centuries and rediscovered from the perspective of functional food products.

## 2. Lukanka, a traditional fermented meat product from the Bulgarian lands

Production of the fermented food products in industrial scales involves combining traditional knowledge with innovative solutions to reproduce quality and benefits of artisanal products in the context of mass production, safety and financial profit. In the last decades, trends in the meat industry can be associated to creations of the strongest and competitive food-processing enterprises [1]. Moreover, implementation of the regulations and directives from the European Union for control of the parameters in the technological processes and safety issues in meat production are some of the most important conditions. Popularization of traditional food products from large-scale industrial production has resulted in the popularity of raw-dried meat products, including well-known Bulgarian sausages such as various types of *sushenitsa*, *babek*, *starets* and *lukanka* (Figure 1). Regarding traditionally well acceptance of the highlighted products by the locals and relatively low average prices per kilogram of 16.04 BGN (approx. 8.00 Euro) that were marketed in Bulgaria, 2020, such products are considered as economically prospective for the production and commercialization by various meat processing enterprises [16].

According to Nilson Bulgaria [17], statistics have shown that raw-dried meat market in Bulgaria contributed to economy with approximately BGN 231.6 million (nearly 116 million Euros) with 6.7% growth from August 2018 to July 2019.

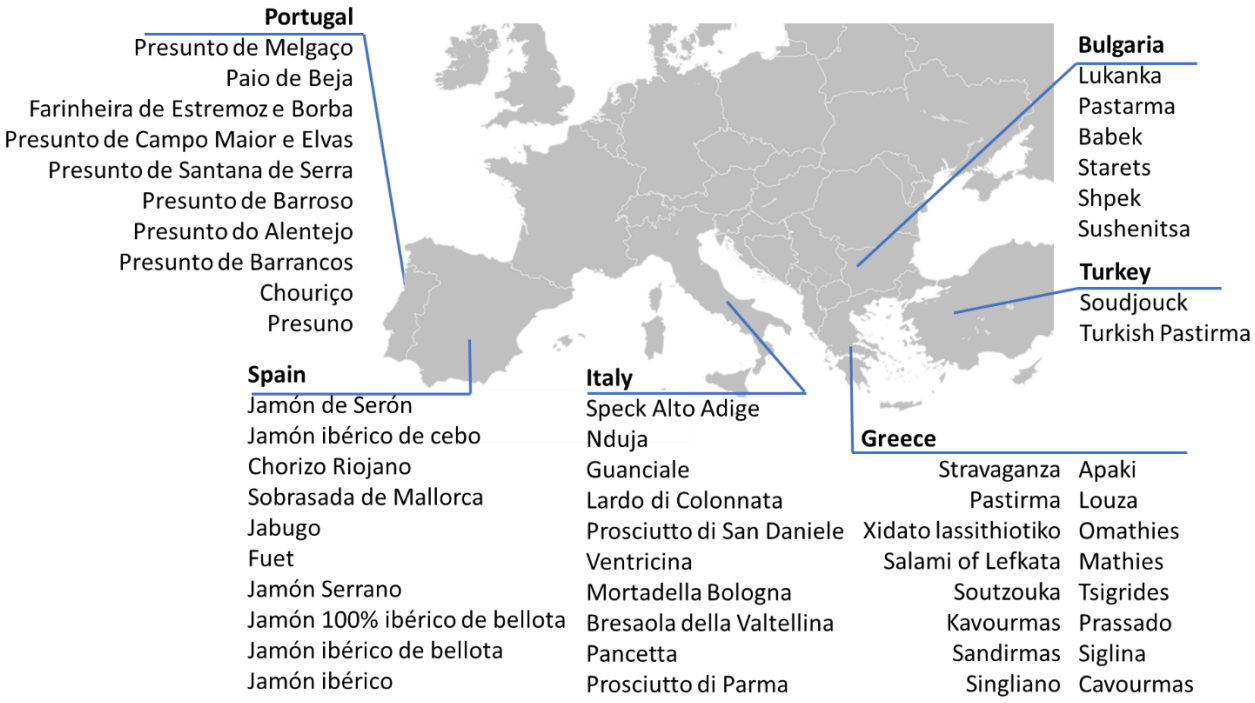


**Figure 1**. Examples of the diversity of the fermented meat products in European Mediterranean countries

It is considered one of the most dynamically developing sectors in 2020 financial year with a specific growth of nearly 23.1% in produced quantities of meat products, as well as 20.3% in financial parameters [17]. One of the best-known dry fermented sausages in Bulgaria is *lukanka*. The origin of name *lukanka* can be traced back to the 19$^{th}$ century. However, most probably this product has been a part of the gastronomic habits for much longer period. Some of the preparation steps for *lukanka* are quite similar to those of other fermented dry meat sausages traditionally produced in the Mediterranean region [18]. However, it is noteworthy that specificity of the primary materials, autochthonous microflora, environmental conditions and technological details in the processing procedures are only a few factors contributing to the final quality and uniqueness of the fermented food products. In 1975, the article "How the Bulgarian sausage was created" by Marinov [18] provided the historical overview on development of *lukanka*, etymology of the name "*lukanka*" linked to the specific way of preparation; in which, an onion was part of the primary ingredients (the word "onion" is incorporated in the *lukanka* name in Bulgarian). In practice, the name "*lukanka*" is verified even onion was later removed from the ingredients.

*Lukanka* is considered an important part of the Bulgarian cuisine heritage (Figure 2). Fermented meat products have long been parts of Mediterranean culture and cuisine and are identified not only by the specificity of their ingredients and preparation processes but also by their shapes and the way they are preserved and consumed. Specificity of *lukanka* can be summarized as a semi-dried fermented sausage with a specific shape (flattened cylindrical), brow-red internal colour and with an external skin enclosed with specific white fungal species. National (Bulgarian) and EU (European Union) regulations describe *lukanka* as a product with relatively low Aw (water activity). Its taste is described as slightly acid and hence recommended to be stored at low temperatures (under refrigeration) in the summer or cold season (winter) at ambient temperature [8]. *Lukanka* includes a granular interior caused by the specific combination of grounded meat and fat used in its production. Based on the traditional recommendations for preparation and standards for industrial production, *lukanka* is manufactured from minced meat (pork and veal), selected spices (e.g. black pepper and cumin) and salt and stuffed into a casing made from dried cow intestine. Artificial casings can sometimes be used to prepare industrial products. In addition to stuffing, technological processes include pressing and shaping, followed by drying for 40-50 d in a well-ventilated area with control of humidity and temperature. After drying, *lukanka* is pressed to acquire its characteristic flat shape, a part of its traditional description [8].

Despite *lukanka* is qualified within these types of fermented meat products, it is a fact that there are competitions between various producers and that producer names are added to the commercial names. For example, the commercial name of *Panagyurska lukanka* is explicit by itself and is derived from the town name of Panagyurishte, where the product was primarily manufactured. Since 1958, industrial production of *lukanka* on large-scales has been started with the Bulgarian state standard of 2589-58 as well as *Panagyurska lukanka* (referring to the place of production), a product which has been unchanged up to date [18]. In accordance with the specifications, *lukanka* is prepared from minced fresh (not frozen) beef, which is sometimes replaced by buffalo and pork meat as well as other additional ingredients and spices stuffed in casings (natural or artificial) with a diameter ranged 50-80 mm, tightly adhered to the filling mass [19,20]. Production of assortments of various fermented salami-type sausages in Bulgaria, including *lukanka*, can be considered as a comparatively novel business practice; however, strongly associated to a traditionally artisanal practice knowledge. Originated from the sources of traditional knowledge of artisans enriched for centuries, the current well-known industrial production has been developed with implem-entation of modern practices [18]. Moreover, several of the production steps were not fully been explained as some approaches are still under comprehensive investigations. Nowadays, detailed proficiency in production of the fermented food products is a critical part for the successful industrial manufacture of specific fermented food product, guaranteeing food safety where a comprehensive deeper understanding of the specificity of technological production is important for providing superior quality of the products to the consumers.

**Figure 2**. A picture of lukanka provided by Radoslav Mladenov, Parvotzvet Ltd, Belogratchik, Bulgaria

Some of the essential control milestones associated to the food safety are fully respected to the good production practices, including severe sanitary and hygienic rules. It is noteworthy that this is one of the critical points in the manufacture of fermented meat products, linked to the fact that these food commodities are consumed raw (not heat treated). Therefore, possibility of specific contaminations in the raw ingredients (e.g. meat, spices and additives) with spoilage or pathogenic microbes should be decreased to the minimal acceptable sanitary requirements. However, appropriate fermentation processes, appropriate starter cultures and correct ripening conditions can contribute to decreasing food pathogens and spoilages and modulating microbiota of the final products. As artisanal products, most of the fermented meat products were generally manufactured during the colder seasons of the year. Nowadays, fermented sausages in most of the countries, including Bulgaria, are manufactured in facilities with appropriate air-conditioned environments [19]. At industrial conditions, temperature in the production facilities (e.g. for primary boning of meat, machine processing technological steps, preparation of stuffing mixtures and packaging processes) needs to be normally below 12 °C [19].

Based on the regulations of the EU from 2012 and amended document from 2020 (Article 53(2) of Regulation EU No. 1151/2012, 2020/C 108/07) and EU No. TSG-BG-01099-AM01-23.7.2019, a *Panagyurska lukanka* should possess the following physicochemical characteristics: maximum water content up to 40% by weight; maximum fat content not exceeding more than 42% by the final weight of the product; a protein content of 28% as a part of the dry matter; maximum salt content of 4.6% by weight and pH nearly 5.2. Commercialization of *Panagyurska lukanka* can be (recommended) as whole sausages; however, it can be cut into slices, packed under vacuum conditions or specific modified gas condition [20]. An important point of preserving gastronomical heritage is to preserve not only quality of the traditional fermented food products but also traditional ingredients and methods of the preparation. Since establishment of the national standards for production, composition and quality requirements have been maintained for over 70 years. Based on the established standards [(assessed in 1983 in technical condition Nr 37-83 approved by the National Agrarian Industrial Union (NAPS), *Panagyurska lukanka* to BDS 2589-83 (National Bulgarian standard) and later EU No. TSG-BG-01099-AM01–23.7.2019)], composition for 100 kg of filling mass for the *lukanka* production must include 60 kg of beef (or buffalo) meat, 20 kg of lean pork, 20 kg of pork breast, 0.3 kg of black or white natural pepper and 0.3 kg of natural cumin. Traditional methods involved in drying and pressing steps of the preparation of the sausages, which are important steps of the *Panagyurska lukanka* production, have been strictly fallowed as in the artisanal process of preparation. The entire production processes, from selecting raw materials and their proportions to pressing, ripening and drying, contribute to the quality of the final products [17,20].

It is well stated in the regulation documents (National Bulgarian standard of BDS 2589-83 and EU No. TSG-BG-01099-AM01-23.7.2019) that *lukanka* can be produced from the fresh (not frozen) meat, normally beef. However it sometimes can be replaced with buffalo and added pork with a final pH of 5.6-6.2 [21]. The meat needs to be removed from bones, chopped and grinded. For better seasoning, spices, salt, permed additives including salts (salt, potassium or sodium nitrate), sugar, ascorbic acid and seasoning (e.g. natural black or white pepper and cumin) and starter cultures are added during the grinding process. In some modifications, the prepared mixture is set overnight for maturation. This primary mixture is stuffed using appropriate equipment into a casing (made of bovine large intestine or ovine caecum as traditional process) or into an artificial casing in the industrial production [21]. Prepared sausages are then arranged on a wooden or metal frames and dried for 2-6 d and then drained at 8-24 °C with relative humidity (RH) of 65-90%. This is dried at 17 °C and 70-85% RH in appropriate natural or air-conditioned drying facilities. During these steps (drying and maturing), *lukanka* is at least two times pressed using flat wooden presses to form its characteristic shape [17,18,20,21]. According to the recommendations, sausages need to set in the presses for 12-24 h and production responsible supervisors visually inspect if the sausages are ready for pressing. *Lukanka* can be pressed once the lower end and the surface are fully dried to the touch and a small quantity of grease is bulging slightly under the casing. In the process of pressing, the product temperature can slightly increase. Drying processes in association to the condensed moisture allows growth of a white noble molds, forming a layer on the *lukanka* surface; a process that is moderated by adding specific starter cultures [18]. Pressing stage of the production must include certain traditional standards. This is a physical stress and sausages must withstand it. However, wooden presses must be able to absorb the moisture during this step. Hence, it is recommended that these presses are made of wood, which is a natural material that can be sanitized and dried. The technological production process for *lukanka* sausages takes at least 20 d and is completed once the product includes its characteristic structure and consistency [17,18,20,21]. Once prepared and properly matured, *lukanka* sausages are subject to final preparations. Their surface is covered by dry white molds, a specific marker that the product is appropriately matured and dried. Lukanka is commercialized in its specific shape of the cylindrical sausages resulted from the pressing process; its aroma and flavor are characterized as slightly salted, with sensorial elements clearly expressed of the used seasoning. The explicit taste and aroma are a combination of meat, complimented by the aroma of the used seasoning,

combination of cumin and black or white pepper. It is noteworthy that *lukanka* cannot be seasoned with garlic, cardamom, allspice and nutmeg (a spice used in preparation of various types of the fermented dry meat sausages). When cut, surface of *lukanka*, which includes an elliptical cross-section compared to other dry cured fermented meat products, has further round or rectangular cross-sections. Color specificity of *lukanka* includes a characteristic brownish-red to red color, resulted from a combination of the meats (beef or buffalo and pork) [17,18,20,21].

## 3. Predominant microorganisms in Bulgarian lukanka

*Lukank*a is a traditionally popular product in Bulgaria as well Balkan Peninsula and South-East Europe countries and is generally valued for its gastronomical qualities. However, a few microbiological studies have been published on natural or fermentation specificity in preparation of *lukanka*. These studies providing information on the microbiota involved in the manufacture of *lukanka* and aspects on their dynamics during fermentation and maturation processes. Dimov et al. [22] assessed presence of various lactobacilli in *lukanka* using whole cell protein (WCP) and DNA based approaches. Totally, 24 isolates from *lukanka* previously identified as *Lb. plantarum*, *Lb. pentosus* and *Lb. paraplantarum* were phylogenetically analyzed using methods based on protein and DNA levels to assess varieties within the *Lactobacillus* species associated with the ripening of *lukanka* [18]. The authors showed a similar clustering of the investigated lactobacilli using the approaches [(WCP electrophoresis (WCPE) and DNA linked methods such as RAPD-PCR, Rep-PCR and ERIC-PCR)]. In addition to assessing biodiversity of lactobacilli in *lukanka*, the authors validated relevance of the highlighted approaches for the ecological analysis of the fermented meat bacterial isolates. They reported that WCPE and Rep-PCR as further appropriate techniques for differentiating especially closely related *Lactobacillus* strains associated to the greater values of the calculated distances within the clusters. Furthermore, it is noteworthy that various LAB strains of similar species were present in the natural fermentation processes of *lukanka*, playing roles in the fermentation processes; thus, verifying the frequently repeated hypothesis that traditional fermented food products are rich sources for the isolation of LAB with various beneficial and functional characteristics.

Stojanovski et al. [23] isolated bacterial species from seven genera : *Lactobacillus, Leuconostoc, Staphylococcus, Enterococcus, Lactococcus, Micrococcus* and *Streptococcus* from diverse industrial lots of *lukanka*, all manufactured by Tandem Company, and showed that they possibly were members of the commonly used industrial starter cultures. Furthermore, more than 200 *Lactobacillus* isolates, collected from the samples covering various stages of *lukanka* production, were analyzed and investigated for their relevance on the production process and their specific technological characteristics. The authors then carried out various sets of experiments covering morphological, cultural, physiological and biochemical analyses [23]. Various end products are formed in food matrices, including meat products, with a large variety of complex substrates, specific polypeptides, organic acids and other fermentable specific compounds. During production of lactic acid (LA), pH changed in the products and different antimicrobials produced were analyzed [23]. In addition to potentially technological characteristics, the authors screened more than 200 isolates for their biopreserving characteristics and highlighted the potential of seven LAB as possible bacteriocin producers, assessing if those strains were appropriate as starter cultures.

Stojanovski et al. [24] preselected 42 specific *Lactobacillus* isolates (all from diverse commercial *lukanka* specimens produced by Tandem Company) and investigated the important technological characteristics. Bacterial growth and acidification characteristics of the selected strains were monitored as well. The commercial quality of the major fermented raw-meat products, including various dry sausages, depended greatly on the fermentative roles of microorganisms participating in fermentation and ripening processes. One of the principal contribution of *Lactobacillus* strains (dependent on their total cell number) in meat fermentation processes includes production of various organic acids and their acidifying characteristics, essential for the conservation of the final products. Changes in pH can include significant roles in changes of peptide profiles and formation of bioactive peptides and free amino acids as well as in safety of the final products. These *Lactobacillus* strains can be involved in lowering of the Aw and pH, two important parameters for the decrease of various spoilage and pathogenic bacteria. Stojanovski et al. [24] selected initially 42 strains in two particular cultures and suggested their uses as starter cultures, showing a significantly better growth. Essential roles in the formation of detailed flavor, pleasant odor, specific color and structure of the fermented sausages are clearly associated to the characteristics of the used raw meats, natural spices, specific microbiota (e.g. LAB) and the technology. During process of the ripening of dry sausages, specific processes (biochemical and physicochemical) normally occur, which alter composition and structure of the meat to form organoleptic properties of the end products. Enzymatic activity of the microbiota, including production of LA, plays an important role in these processes [25,26]. Specific organoleptic properties of the final products are clearly linked to the potential of starter cultures to produce and express various enzymes.

Stojanovski et al. [27] used API Zym kit (BioMerieux, France) to assess production of various enzymes of the previously isolated lactobacilli from *lukanka*. Enzymatic activity of the studied strains ($n$ = 31), belonged to *Lb.*

*plantarum, Lb. sakei* and *Levilactobacillus brevis* and isolated from various stages of *lukanka* fermentation process, was investigated. It was observed that a majority of the studied isolates presented relatively low lipolytic activities. Moreover, productions of phosphatase and β- galactosidase were recorded for a few strains. No specific amidase activity was observed that varied within the bacterial cultures. High levels of β-glucosidase, α-galactosidase, β-glucuronidase, α-manosidase and N-acetyl-β-glucosaminidase enzymatic activity were recorded for almost all of the strains. However, it is a fact that complex biochemical processes that occur during the ripening stage of the dry meat production processes are resulted from the specific interactions of the remaining enzymes from the muscle and fat tissues, strongly predisposed by the action of the specific bacterial enzymes including that of LAB [27]. Artisanal fermented meat products can be considered as an important source of LAB and regarded as a delivery vector of LAB to the consumers. It has been verified that *lukanka* can include various LAB; some can be associated to specific beneficial and probiotic characteristics [8]. Previously, Todorov et al. [8] studied specific LAB isolated from *lukanka* with objectives to assess their safety and specific probiotic/beneficial characteristics. The selected LAB [8] were assessed for several technol-ogical parameters (including multiplication at various temperatures, role of pH and specific proteolytic activity). Some of these LAB seemed as good candidates for the starter cultures. Moreover, assessment of relative safety factors showed that presence of specific genes linked to the expression of virulence factors, production of BA and vancomycin resistance were present only in a few strains of the LAB [8]. Additionally, expression of antimicrobial peptides (bacteriocins) and presence of appropriate genes associated to bacteriocin production were prevalent in the studied strains, enabling them to play roles in biopreservation of the final products. Todorov et al. [8] highlighted a very strong activity of the isolated LAB against *L. mono-cytogenes.* However, the assessed bacteriocins did not include inhibitory activity against closely related bacteria of *Lactobacillus* spp., *Lactococcus* spp., *Enterococcus* spp. and *Pediococcus* spp. [8].

## 4. Roles of the starter cultures in the production of fermented meat sausages

Starter cultures not only in fermented meat products but also in other food products play important roles in moderating fermentation processes and initiating fast lowering of the pH, which results in acidification of the raw meats batter and hence leads to the formation of desired sensory qualities in the final products. During the last decade, beneficial and functional bacterial cultures have been investigated as effective starter cultures with industrial (technological) or nutritional and health promote characteristics. It has been hypothesized that well selected starter cultures with functional characteristics can offer additional benefits, compared with current traditional starter cultures. These starter cultures can be used not only for technologically important processes in preparation of the fermented meat products but also for the production of food commodity with tastier, safer and healthier characteristics [7]. Examples include microbial strains that can contribute actively to the development of aroma compounds as well as health-promoting metabolites such as bacteriocins or other antimicrobial molecules and postbiotics, produce cured meat color, microorganisms with probiotic characteristics and increased safety characteristics with no produced BA and toxic compounds [7,13,14]. Wide variety and quantity of the primary raw materials (meats and other ingredients) and differences in fermentation, drying, maturation and storage create sensory characteristics of the end products diversely and uniquely. Various countries have their own traditional fermented sausages, often protected as national heritage products. To contribute to improvement of the quality and safety of these products, similar problems occur Worldwide. These problems include standardization of the production processes and development of the starter cultures that facilitate the fermentation process in the desirable direction to produce consistent quality of the final products and guarantee the safety for the consumers [22,23,28]. Fermented meat products are generally made with starter cultures, including specific coagulase-negative cocci and selected LAB such as *Lb. sakei*, *Lb. curvatus* and *Lb. plantarum* as well as yeasts [29]. The major technological contribution to the fermentation processes of LAB is generally linked to decreased pH of the food matrix associated to the production of LA. Decreased pH is needed for the coagulation of fibrillar proteins, improving the quality and more specifically the firmness and cohesiveness of the final products and allowing for easier cutting [30]. Montel et al. [25] have suggested that LAB can be involved in the essential processes of generating flavors by metabolizing generated acetic acid, formic acid and succinic acid during carbohydrates metabolism. Moreover, LAB can play vital roles in inhibition of the growth of foodborne pathogenic and spoilage bacteria. This can be attributed to the ability of LAB strains to produce various organic acids or other specific antimicrobial metabolites, including hydrogen peroxide, diacetyl and antimicrobial peptides (including bacteriocins) [31,32]. Bacteriocins have been verified to provide added controls against various pathogens in fermented sausages [14,33].

## 5. Micro flora of the naturally fermented raw-dried sausages

Microbiological analyses of traditionally prepared raw-dried sausages from Portugal, Spain, Italy and Greece revealed the presence of diverse microorganisms. These included aerobic, mesophilic and psychotropic micro-organisms, spore-forming bacteria, LAB, halophilic bacteria,

coagulase-negative cocci, enterococci and molds; from which, two groups of microorganisms, including LAB and coagulase-negative staphylococci, are considered technologically important for the fermentation and maturation of dry sausages [34-37]. An overview of the literature shows that these predominate through the fermentation. Lactobacilli (especially homofermentative species) account for the majority of the population with *Lb. sakei*, *Lb. curvatus*, *Lb. casei* and *Lb. plantarum* as the most commonly identified species. Moreover, *Lb. alimentarius* has been detected in Spanish, *Lb. rhamnosus* in Greek and *Lb. brevis* and *Lb. versmoldensis* in Scandinavian sausages. Representatives of the genera of *Lactococcus*, *Pediococcus* and *Leuconostoc* were reported as well [34-36]. Additionally, *Staphylococcus (S.) xylosus* was the most commonly isolated species from the group of coagulase-negative staphylococci reported for various types of salamis, associated majorly with the Italian products [29] and Spanish sausages [36]. *S. saprophyticus* and *S. carnosus* were the dominant species in traditional Greek sausages [26,38,39]. Species such as *S. simulans, S. epidermidis, S. haemolyticus, S. warneri, S. equorum, S. gallinarum, S. cohnii* and *S. pasteuri* less frequently isolated from various fermented sausages [26,38,39].

## 6. Micro biota of the fermented raw-dried sausages prepared with starter cultures

Raw-dried sausages are usually produced using a combination of LAB, most commonly various lactobacilli and coagulase-negative micrococci isolated from meat products, and selected based on the technological aspects inherent to the two taxa [40]. The first attempts to use microbial starter cultures for the production of fermented meat products occurred in USA in 1940, when pure cultures of representatives of a group of lactobacilli were used. Later in 1955, use of strains, belonging to *Micrococcus caseolyticus*, was industrially started in Finland. In Germany, strains belonged to *Pediococcus cerevisiae* were used in the production of raw-dried sausages [40]. In Bulgaria, a starter culture was used to produce *Panagyurishte* sausages and production by DSO Rodopa was reported for the first time in 1972 [36]. Currently, Genesis starter cultures are produced in Bulgaria for making raw-dried sausages, which are concentrated and lyophilized cultures of the selected strains of micrococci, staphylococci and pediococci for direct uses in the meat masses [22].

## 7. Technologically significant micro-biota in the traditional technology for sausage production

Sausages produced by traditional technologies include more abundant and diverse microorganisms than that sausages produced by starter cultures do. This is well represented in the population of LAB and members of the *Micrococcaceae* family (Table 1). Therefore, the number of viable enterococci present in the minced meats during filling in sausages that are made from starter cultures is 1-2 logarithmic numbers of magnitude higher than that in traditionally prepared sausages [41].

**Table 1**. Microbiota of *lukanka* and other dry fermented meat sausages

| Bacterial species | Origin of isolation | References |
|---|---|---|
| Lactobacilli | *lukanka*, Bulgaria | [41] |
| *Micrococcaceae* | *lukanka*, Bulgaria | [41] |
| *Lactobacillus* sp. | *lukanka*, Bulgaria | [41] |
| *Enterococcus* sp. | *lukanka*, Bulgaria | [41] |
| *Micrococcaceae* | *lukanka*, Bulgaria | [41] |
| *Lactiplantibacillus plantarum* | *lukanka*, Bulgaria | [42] |
| *Levilactobacillus brevis* | *lukanka*, Bulgaria | [42] |
| *Latilactobacillus sakei* | *lukanka*, Bulgaria | [42] |
| *Lactiplantibacillus plantarum* GPL3 and *Debaromyceshasenii* (starter culture) | *lukanka*, Bulgaria | [42] |
| *Lactobacilli plantarum* | Fermented sausages, USA | [43] |
| *Pediococcus cerevisiae* | Fermented sausages, USA | [43] |
| Streptococci | Fermented sausages, USA | [43] |
| Lactobacilli | Dry cured sausage, Dacia, Romania | [44] |
| *Micrococcaceae* | Dry cured sausage, Dacia, Romania | [44] |
| Lactic acid bacteria | Sausages (spontaneous fermentation), Brazil | [45] |
| *Micrococcacae* | Sausages (spontaneous fermentation), Brazil | [45] |
| *Staphylococcus xylosus* | Sausages (spontaneous fermentation), Brazil | [45] |
| *Staphylococcus carnosus* | Sausages (spontaneous fermentation), Brazil | [45] |
| *Staphylococcus saprophyticus* | Sausages (spontaneous fermentation), Brazil | [45] |
| *Lactiplantibacillus plantarum* | Traditional Chinese sausages | [46] |
| *Limosilactobacillus fermentum* | Traditional Chinese sausages | [46] |
| *Lactiplantibacillus pentosus* | Traditional Chinese sausages | [46] |

Their number increases in the draining stage, reaching to nearly $10^8$ CFU $g^{-1}$ for LAB and micrococci and nearly $10^6$ CFU $g^{-1}$ for enterococci and is constant until the evacuation stage. The number of LAB in the starting minced meats for the preparation of traditional sausages is $7.5 \times 10^3$ CFU $g^{-1}$ and reaches higher values before the first pressing, exceeding those of sausages prepared by the starter cultures ($7.35 \times 10^8$ CFU $g^{-1}$ compared to $1.03 \times 10^8$ CFU $g^{-1}$, respectively). As the product is evacuated, their number decreases and has almost equal values in the two types of sausages [41,44,45]. Micrococci population also increases after the draining stage but the increase in their number is constant in the final products; as it was higher in the traditionally prepared products. Furthermore, differences were observed between the representatives of micrococci between the spontaneous fermentation and starter cultures. In products prepared with starter culture under standardized conditions, almost only mannitol-negative micrococci were seen, while mannitol-positive ones were occasionally isolated. In contrast, mannitol-positive micrococci were dominated in the traditional sausages produced by spontaneous fermentation, most of which included the ability to form mannitol acid aerobically and anaerobically [41,44,45]. Differences were also observed in enterococci of the two types of sausages, traditionally made with spontaneous fermentation and industrially made with starter cultures. In standardized sausag-es, representatives of the *Enterococcus* spp. developed rapidly and abundantly (from $6.25 \times 10^3$ CFU $g^{-1}$ to $7.9 \times 10^6$ CFU $g^{-1}$), following the growth dynamics of the components of the starter cultures. In the traditional sausages (spontaneous fermentation process), enterococci population was reasonably low ($8.5 \times 10^2$ CFU $g^{-1}$) but slowly increased upon grinding of the minced meats ($8.5 \times 10^2$ CFU $g^{-1}$) and reached a maximum number of nearly $10^5$ CFU $g^{-1}$ in intermediate stages with a subsequent gradual decrease to $2.28 \times 10^4$ CFU $g^{-1}$ at the final stage of production. Based on the literature, the final number for enterococci in various fermented meat sausages was $10^5$-$10^6$ CFU $g^{-1}$ at the end of fermentation. Enterococci can produce ammonia and other amines, which possibly create a certain taste in the final products [39,47-50].

In naturally fermented products, the number of yeasts is much higher in the initial minced meats and through the fermentation process and their dynamics follow those of fermenting bacteria. In standardized sausages, the yeast population was reported unstable in number and included significant decreases in their quantity. However, a limited information is available in the literature associated to the yeasts in fermentation of sausages. Thus, effects of their presence in fermented sausages are unclear. According to some authors, ability of yeasts to ferment sugars with their proteolytic and lipolytic activities results in production of low molecular weight aromatic compounds that are involved in formation of the aromatic-flavor raw-dried sausages [51-53]. According to other authors, yeasts can be considered as undesirable components in the meat microflora due to their high lipase production [36,39]. The last stage of drying of the fermented sausages is characterized by the predominant development in the two product types of LAB (mostly lactobacilli), micrococci, enterococci and yeasts [41-46]. Authors identified and characterized bacterial species with proteolytic characteristics associated to the breakdown of meat proteins into peptones, peptides, free amino acids (FAA) and ammonia, causing deterioration or spoilage. In previous studies, proteolytic bacterial species were shown to be represented by many aerobic and anaerobic microorganisms with predominantly proteolytic activities. Immediately after the filling stage, changes in the oxygen content led to predominant developments of LAB and micrococci and generally non-spore-forming proteolytic microorganisms [36]. In the next stages, growth of microorganisms was assessed; in which, no proteolytic activities were recorded and their presence were insignificant [36]. In fact, CNC and LAB can contribute significantly to meat maturation as their synergistic effects lead to good characteristics such as color, taste, aroma of meat products [29,47,54-56]. Based on the result of systematic studies on *Panagyuriska lukanka*, coagulase and hemolysis-negative representatives of the *Micrococcaceae* family have been isolated [41]. Moreover, representatives of the *Enterococcus* genus were isolated. However, it is noteworthy that the two bacterial groups were catalase negative [41]. The predominant numbers of isolates were representatives of the LAB [41,42].

## 8. Conclusion

Mediterranean region is traditionally associated to the production of various fermented dry sausages as a part of their culinary heritages for centuries, each of which includes its own traditional preparation, specific ingredients and microbiome. These culinary heritages are essential parts of cultural and gastronomic heritages of the region. From the perspective of the microbiology, these are rich sources of isolation of functional, beneficial and biotechnological microbial cultures. *Lukanka*, a Bulgarian fermented dry-meat product, is one of these heritages, highly appreciated for its gastronomical qualities not only in Bulgarian lands but also in large parts of the Europe. The LAB isolated from *lukanka* have been demonstrated with highly technological and specifically beneficial characteristics, making them appropriate to use as starter cultures for the fermentation of beneficial and functional meat products with extremely appreciated gastronomic and health benefits.

## 9. Authors Contributions

Conceptualization, I.V.I. and S.D.T.; data research, T.V., C.M.D., I.V.I, P.M. and S.D.T.; writing-original draft preparation, P.M., I.V.I. and S.D.T.; writing-review and

editing, I.V.I., C.M.D., M.T.L. and S.D.T.; visualization, S.D.T.; supervision, I.V.I. and S.D.T.; project administration, S.D.T.; funding acquisition, I.I., T.V. and S.D.T. All authors have read and agreed to publish this version of the manuscript.

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## 12. Conflict of Interest

The authors report no conflicts of interest.

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# لوکانکا، به عنوان سوسیس نیمه خشک سنتی تخمیری بلغاری: نقش کشت‌های باکتریایی در ویژگی‌های فناورانه، ایمنی و سلامتی‌بخش آن

**سوتوسلاو دیمیتروف تودوروف[1*]، کلاریزا می دیوسو[2]، مین تزه لیونگ[3]، تونکا واسیلوا[4]، پنکا مونچوا[5]، ایسکرا ویتانوا ایوانووا[5]، ایلیا ایلیف[4]**

۱- گروه همگرایی پیشرفته، دانشگاه جهانی هاندونگ، پوهانگ، Gyeongbuk 37554، جمهوری کره.
۲- مؤسسه علوم زیستی، دانشگاه فیلیپین لوس بانوس، لاگونا، فیلیپین.
۳- دانشکده فناوری صنعتی، دانشگاه ساینس مالزی، ۱۱۸۰۰ پنانگ، مالزی.
۴- گروه بیوشیمی و میکروبیولوژی، دانشکده زیست شناسی، دانشگاه پلوودیو "Paisii Hilendarski"، پلوودیو، بلغارستان.
۵- دانشگاه صوفیه "St. Kliment Ohridski"، دانشکده زیست شناسی، گروه میکروبیولوژی عمومی و صنعتی، صوفیه، بلغارستان.

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- *لوکانکا*
- کشت‌های آغازگر

***نویسنده مسئول**
**سوتوسلاو دیمیتروف تودوروف**
گروه همگرایی پیشرفته، دانشگاه جهانی هاندونگ، پوهانگ، Gyeongbuk 37554، جمهوری کره.
تلفن: ۸۲-۱۰-۳۴۹۰۳۱۵۲ +

پست الکترونیک:
slavi310570@abv.bg

## چکیده

**سابقه و هدف:** فرآیندهای تخمیر، دود کردن و نمک اندود کردن مواد اولیه، بخشی از انواع فرآیندهای نگهداری هستند که برای تولید محصولات نهایی در کشورهای جنوب اروپا ضروری به‌حساب می‌آیند. در اصل، تخمیر فرآورده‌های گوشتی برای نگهداری طولانی و ایمن استفاده می‌شود. به هر حال، در حال حاضر برای دستیابی به طعم، عطر، رنگ و ساختار سالامی مطلوب مصرف کننده، درنظر گرفتن ویژگی‌های گوارشی فرآورده‌های گوشتی تخمیری ضروری است. تاکید بر ویژگی‌های گوارشی است که در نتیجه انواع اختلاط گوشت خام، ادویه‌های خاص و میکروبیوتای طبیعی یا فرآیندهای تخمیر با استفاده از کشت‌های آغازگرهای خاص حاصل می‌شود. هدف از این بررسی، ارائه اطلاعات در مورد منشاء لوکانکا، یک فرآورده نیمه خشک تخمیری بلغاری، دانش در مورد ویژگی میکروبیولوژیکی محصول و نمونه‌هایی از ویژگی‌های مفید مرتبط با باکتری لاکتیک اسید جدا شده از لوکانکا بوده است.

**یافته‌ها و نتیجه‌گیری:** کشت آغازگر گوشت معمولاً شامل هفت گونه باکتریایی متعلق به جنس‌های *لاکتوباسیلوس*، *لوکونوستوک*، *استافیلوکوک*، *انتروکوک*، *لاکتوکوک*، *میکروکوک* و *استرپتوکوک* می‌باشد. انواع گوناگون باکتری لاکتیک اسید از لوکانکا جدا شده است و از نظر ایمنی و ویژگی‌های سلامتی‌بخش ارزیابی شده‌است. در طول رسیدگی فرآورده‌های تخمیری گوشتی، در نتیجه برهمکنش بین کشت‌های آغازگر باکتری، آنزیم‌های باقی‌مانده در ماهیچه‌ها و بافت‌های چربی و آنزیم های باکتریایی فرآیندهای پیچیده تخمیری رخ می‌دهد. نقش ویژگی‌های مفید باکتری‌های لاکتیک اسید جدا شده از لوکانکا به‌عنوان ضرورتی برای مشخصه‌دار کردن لوکانکا به‌عنوان یک فرآورده غذایی فراسودمند[1] بررسی شده‌است. فرآورده گوشتی تخمیر شده سنتی سامانه‌های اکولوژیکی خاصی هستند که در آن فعل و انفعالات بین سویه‌های مختلف باکتریایی و ماتریس‌های غذایی منجر به تشکیل ویژگی‌های منحصر به فرد محصول نهایی می‌شود. با آنالیز سوسیس‌های گوشتی تخمیر شده سنتی از دیدگاه علم قرن بیست و یکم، می‌توان به این غذاها به عنوان نمونه‌هایی از فرآورده‌های غذایی فراسودمند اشاره کرد که مزایای تغذیه‌ای، گوارشی و مفیدی را برای مصرف‌کنندگان خواهد داشت.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[1] functional food product