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Genetic and biochemical characterization of *Lacticaseibacillus rhamnosus* MDC 2012 and its potential in processing of raw pork meat

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ABSTRACT

Background: Pork is an excellent source of essential amino acids, particularly ones containing sulfur, due to its abundance of proteins with high biological value. During meat maturation, amino acids and peptides undergo transformations that directly influence flavor. The meat-fermenting strain *Lacticaseibacillus rhamnosus* MDC 2012 (previously *Lactobacillus rhamnosus* MDC 2012) offers potential to enhance proteolysis and improve nutritional and sensory qualities.

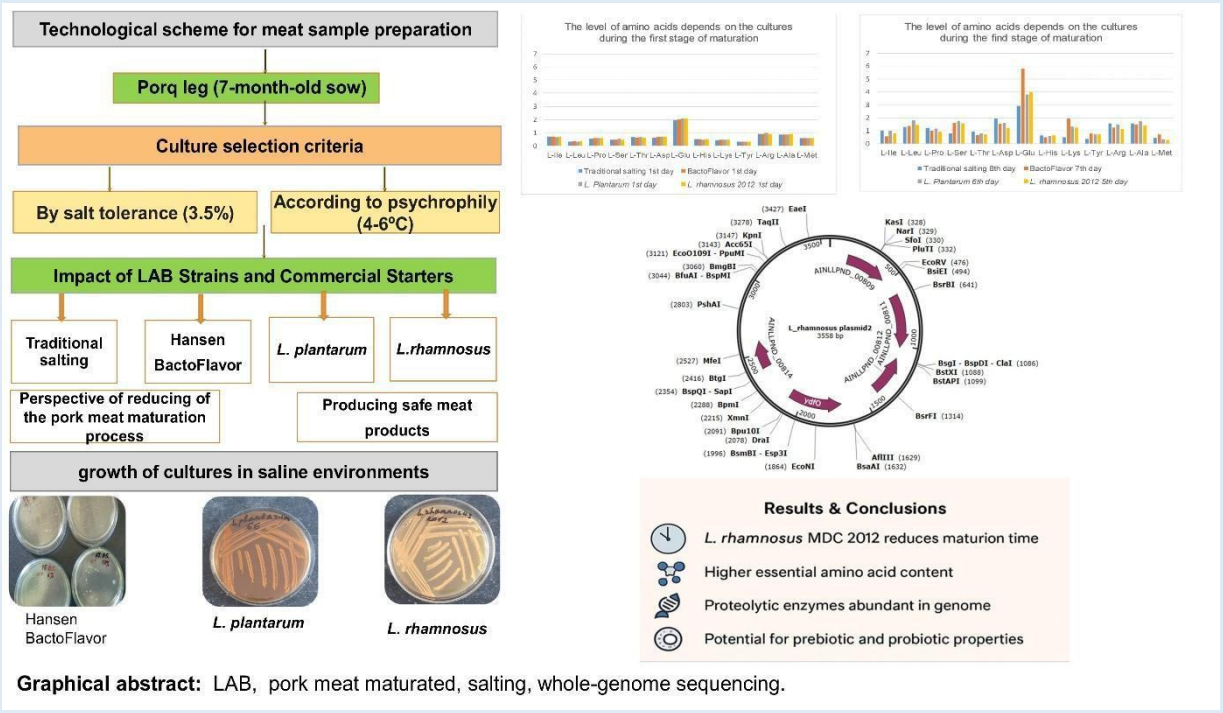
Objective: This study aimed to investigate the effect of *L. rhamnosus* MDC 2012 on pork amino acid composition during salting and maturation, and to interpret the observed changes using genomic data of the strain.

Methods: Amino acid composition of pork was determined using Gas Chromatography/Mass Spectrometry on a Bruker Scion SQ 450 GC-MS device (USA), following the manufacturer's protocol. Whole-genome sequencing was performed with Oxford Nanopore Technologies (UK) on a Flongle R10.4.1 flow cell. High-molecular-weight DNA was isolated using the phenol-chloroform extraction method. Genome analysis focused on proteolytic enzymes relevant to protein degradation.

Results: Application of *L. rhamnosus* MDC 2012 reduced the pork maturation period from 8 to 5 days. Essential amino acids increased significantly, including L-glutamic acid (2.93 → 4.00 %), L-lysine (0.48 → 1.23 %), L-serine (0.83 → 1.57 %), and L-leucine (1.30 → 1.43 %). The marked rise in L-glutamic acid is especially important for the development of brothy flavors. Enhanced L-lysine and L-leucine content improved the biological value of pork. Genomic sequencing revealed more than 21 proteases in *L. rhamnosus* MDC 2012, which likely contributed to the observed proteolysis and quality enhancement.

Conclusion: Use of *L. rhamnosus* MDC 2012 during pork maturation shortened the processing time while enriching essential amino acids. The strain's proteolytic enzymes support the generation of peptides and flavor compounds, improving both nutritional quality and sensory attributes. These findings highlight the potential of *L. rhamnosus* MDC 2012 as a functional starter culture with practical applications in meat production.

Keywords: fermented pork meat, salting, amino acid, *Lacticaseibacillus rhamnosus*, whole-genome sequencing.



Graphical abstract: LAB, pork meat maturated, salting, whole-genome sequencing.

INTRODUCTION

Meat is a rich source of high-quality protein containing all indispensable amino acids (IAAs), particularly those containing sulfur, making it a fundamental component of the human diet. In addition to its nutritional value, meat also contains naturally occurring compounds such as biogenic amines (BAs), which play essential roles in human metabolism as building blocks of proteins, growth factors, or stabilizers of nucleic acids, while also significantly contributing to the sensory characteristics of the final product [1].

Specific free amino acids released during meat aging, particularly L-glutamic acid and L-aspartic acid, are key contributors to umami flavor. This "brothy» or «savory" flavor enhances sensory perception and complements other taste attributes, thereby influencing consumer acceptance and the overall quality of meat products. Integrating the study of these amino acids

with the investigation of microbial fermentation provides valuable insight into how starter cultures can modulate both nutritional and sensory properties simultaneously.

Pork is the most widely consumed animal protein source worldwide and remains the most commercially traded meat in the global market. Biochemical processes occurring during the handling, processing, and storage of animal-derived raw materials have a profound impact on the quality of meat products, including dry-cured meat, fresh meat, cheese, and fish [2]. The extent of hydrolytic degradation of proteins, ribonucleotides, and tissue structures is largely influenced by both environmental conditions and microbial enzymatic activity.

During the conversion of muscle into meat and its subsequent aging, proteolytic activity plays a critical role in determining key quality parameters such as tenderness, flavor development, the release of bioactive peptides, and the water-holding capacity of meat [3-5]. Understanding and controlling these proteolytic processes is essential for the optimization of high-quality meat production.

In this context, creatine plays a pivotal role as a major component of the muscle energy transfer system. In pigs, creatine is synthesized primarily in the liver, kidneys, and pancreas from the amino acids glycine, arginine, and methionine [6-8]. The amidino group of arginine is transferred to glycine by the enzyme L-arginine: glycine amidinotransferase (EC 2.1.4.1), forming guanidinoacetate, which is then methylated by S-adenosylmethionine to produce creatine. Up to 95–98% of creatine is stored in skeletal muscle as phosphocreatine, where it serves as a readily available energy reserve.

Postmortem, phosphocreatine helps delay glycogen metabolism and lactate formation, slowing the pH decline during meat conversion and thereby improving meat quality attributes such as pH stability, water-holding capacity, color, and reduced drip loss. These improvements are also observed in pigs carrying the RN^- and RYR1T genes [9]. However, it is important to note that creatine in meat may also act as a precursor of heterocyclic aromatic amines (HAAs), compounds that can form during thermal processing and are considered potentially mutagenic to humans.

In meat processing, lactic acid bacteria (LAB) play a crucial role as starter cultures, enhancing proteolysis, lipolysis, and fermentation reactions [10-11]. These microbial transformations contribute to improved sensory and nutritional attributes, stable aroma, and desirable color [12]. However, some LAB strains also possess decarboxylase enzymes that convert amino acids into biogenic amines. This enzymatic activity, dependent on pyridoxal-5-phosphate as a cofactor, may lead to excessive BA accumulation, posing health risks and affecting product safety and acceptability. Among promising LAB candidates, *Lacticaseibacillus rhamnosus* MDC 2012 (formerly *Lactobacillus rhamnosus* MDC 2012*)* stands out for its potential use in fermented pork products. Whole-genome sequencing of this strain revealed a wide array of genes encoding proteolytic enzymes, supporting its ability to enhance amino acid release and reduce fermentation time. These characteristics may improve the flavor and nutritional properties of meat while mitigating undesirable BA accumulation.

The whole-genome sequence and demonstration of a low frequency of undesirable characteristics—such as plasmids, prophages, antibiotic resistance genes, and virulence factors—along with frequent bacteriocin production, facilitate the identification of the strain and its potential food fermenting properties. These traits support the classification of this species as a good candidate for use as a probiotic [13]. Nowadays, Next Generation Sequencing (NGS) is a standard procedure for whole bacterial genome sequencing [14]. Sequencing the complete genome of probiotic bacteria is more successful when long-read methods, such as PacBio or Oxford Nanopore, are used [15-16].

This study aims to investigate the effect of *L. rhamnosus* MDC 2012 on the amino acid profile and BA dynamics during pork fermentation. In addition, the genomic potential of the new strain used is evaluated to explain the observed biochemical transformations and to assess its applicability in the development of functional, high-quality meat products.

MATERIALS AND METHODS

Materials: The pork leg was obtained from a supermarket, and the N5 approval document is available. One of the primary and key stages of the production process is the safe handling and transportation of raw meat; therefore, all procedures are regulated by Regulation 034/2013 "On Safety of Meat and Meat Products'' Technical Regulations of the Customs Union (TR CU) https://mineconomy.am/page/444. In accordance with the technical regulations, raw meat produced and sent to the production line must originate from a slaughterhouse and be accompanied by the necessary documents or certificates https://www.arlis.am/DocumentView.aspx?docid=118821.

Strains and fermentation conditions: The availability of such documents confirms the safety of manufacturing and transportation of the raw meat. The experimental material used in this study was pork meat derived from the hind leg of 7-month-old sows. The samples were processed under controlled laboratory conditions to evaluate the effects of selected starter cultures on the biochemical transformations and amino acid composition of pork during salting and maturation. Microbial strains were selected based on two physiological properties: tolerance to NaCl up to 3.5% and psychrophilic growth, with activity sustained at temperatures ranging from 4°C to 6°C. The following starter cultures were selected for the study: BactoFlavor® BFL-T0 (Hansen, Denmark): a widely used commercial starter culture comprising *Pediococcus pentosaceus* and Staphylococcus carnosus. *Lactobacillus plantarum* 66 MDC 9619 and *Lacticaseibacillus rhamnosus* MDC 2012. The latter two strains were obtained from the Microbial Depository Center (MDC) of the SPC "Armbiotechnology" of the National Academy of Sciences of the Republic of Armenia (NAS RA). This institution is officially registered in the World Data Center for Microorganisms (WDCM) database (https://armbiotech.am; www.wfcc.info). A control group using traditional dry salting (without starter cultures) was included to serve as a baseline for comparative analysis. All samples were salted and matured in standardized conditions (temperature, humidity, and duration) appropriate for evaluating the fermentation process. The experimental groups were inoculated with selected starter cultures and matured alongside the control group for comparative analysis, which included the determination of creatinine and amino acid content in pork meat samples.

The determination of creatinine and amino acids content in pork meat samples was carried out at the beginning of the meat maturation period—on the 1st and 4th days of refrigerated storage, as well as in the matured meat. Maturation was considered complete on the 8th day under traditional salting conditions, on the 7th day under the Hahnssen starter culture, on the 6th day with *Lactobacillus plantarum* 66, and on the 5th day in the presence of *L. rhamnosus* MDC 2012 0 [17].

Determination of creatinine: Creatinine content was measured using a Jenway™ 7315 UV/Visible Single Beam spectrophotometer (Jenway™ 7315, Stone, Staffordshire, United Kingdom).

Determination of amino acids: The amino acid composition of pork processing raw materials was determined using Gas Chromatography/Mass Spectrometry on a Bruker Scion SQ 450 GC-MS device (USA), following the manufacturer's protocol.

Sequencing: Whole-genome sequencing was performed following the recommendations of Oxford Nanopore Technologies (UK), using a Flongle R10.4.1 flow cell and the ligation sequencing kit SQK-LSK114, according to the protocol specified in the g-dna-by-ligation-sqk-lsk114-document (document: Flongle-en- GDE_9161_v114_revY_30Jan2025.pdf). High-molecular-weight, high-purity DNA was isolated using a classic phenol-chloroform extraction method. The raw sequencing data were processed using the Oxford Nanopore Technologies EPI2ME (EPI2ME Desktop V5.2.3) Bacterial Genomes workflow.

RESULTS AND DISCUSSION

Meat creatinine profile during pork processing: The results of our study indicate that the use of different starter cultures—specifically BactoFlavor® (*Pediococcus pentosaceus, Staphylococcus carnosus, Subs. utilis*), *Lactobacillus plantarum* 66 MDC 9619, and *Lacticaseibacillus rhamnosus* MDC 2012 (formerly *Lactobacillus rhamnosus*) significantly affects the dynamics of creatinine content in pork meat during maturation, in comparison to traditional salting. As shown in Table 1, creatinine levels decreased consistently during maturation, mainly due to a reduction in pH and the activation of biochemical processes involved in protein degradation. Creatinine is a nitrogenous extractive compound that serves as a precursor in the formation of flavor and aroma-contributing substances in pork meat.

Table 1. Creatinine content depending on starter cultures and maturation stages of pork.

Creatinine content during pork maturation days (mmol/g)			
Type of salting	Maturation phase		
	1st day	4thday	Matured
Traditional salting	4.47±0.10	3.48±0.08	2.43±0.04
BactoFlavor®	6.13±0.37	4.80±0.06	2.70±0.05
L. plantarum	4.15±0.10	3.51±0.14	1.94±0.08
L. rhamnosus	6.80±0.10	3.80±0.05	2.15±0.10

On the first day of maturation, 24 hours after salting, the highest creatinine concentrations were observed in samples treated with BactoFlavor® and *L. rhamnosus* MDC 2012. However, by the final stage of maturation, a more pronounced reduction in creatinine levels was observed in samples inoculated with *L. rhamnosus* MDC 2012 and *L. plantarum 66* MDC 9619, suggesting higher metabolic and enzymatic activity by these strains.

Overall, the data suggest that the application of starter cultures accelerates proteolysis and the transformation of nitrogenous compounds. In particular, the rapid decrease in creatinine observed in the presence of *L. rhamnosus* MDC 2012 indicates its strong proteolytic potential, which may enhance maturation rate and improve the flavor intensity of the final meat product.

Meat amino acid profile during pork processing: In the present study, the samples were analyzed for 13 amino acids (isoleucine, leucine, proline, serine, threonine, aspartic acid, glutamic acid, histidine, lysine, arginine, alanine, methionine), as presented in Table 2.

Table 2. Amino acid content depending on starter cultures and maturation stages of pork 100 g/%.

Amino acid	Type of salting											
	Traditional salting			BactoFlavor®			*L. plantarum* 66 MDC 9619			*L. rhamnosus* MDC 2012		
	Maturation phase											
	Start	4thday	8thday	Start	4thday	7thday	Start	4thday	6thday	Start	4thday	5thday
L-Ile	0.70	0.78	1.01	0.71	0.65	0.56	0.69	0.81	0.97	0.70	0.78	0.81
L-Leu	0.32	0.39	1.30	0.36	0.64	1.37	0.34	1.15	1.77	0.36	0.50	1.43
L-Pro	0.57	0.61	1.20	0.60	0.63	0.98	0.59	1.03	1.17	0.59	0.61	0,92
L-Ser	0.47	0.49	0.83	0.50	1.55	1.58	0.55	1.71	1.76	0.50	1.54	1.57
L-Thr	0.68	0.81	0.93	0.65	0.68	0.68	0.68	0.78	0.82	0.65	0.68	0.72
L-Asp	0.65	0.70	1.94	0.69	0.88	1.50	0.73	0.84	1.58	0.71	0.79	1.20
L-Glu	1.94	2.08	2.93	2.03	2.46	5.79	2.10	2.20	3.82	2.09	2.41	4.0
L-His	0.52	0.58	0.65	0.52	0.50	0.50	0.50	0.58	0.60	0.52	0.58	0.64
L-Lys	0.44	0.47	0.48	0.48	0.67	1.93	0.50	0.71	1.33	0.48	0.52	1.23
L-Tyr	0.32	0.35	0.37	0.35	0.66	0.80	0.35	0.68	0.75	0.35	0.68	0.70
L-Arg	0.90	1.01	1.54	0.91	1.14	1.25	0.99	1.25	1.47	0.91	1.05	1.13
L-Ala	0.85	0.88	1.54	0.85	1.10	1.48	0.85	0.85	1.75	0.89	0.93	1.42
L-Met	0.60	0.55	0.44	0.6	0.68	0.72	0.61	0.55	0.35	0.60	0.42	0.29

According to the table, the amino acid content varies depending on the starter cultures and maturation stage. It is well known that proteolysis results in the generation of large peptides, which are in turn degraded to oligopeptides and then to free amino acids (FAAs), and this is one of the most important biochemical changes occurring during the aging of dry-cured fermented meat products [18-19]. In addition to their biological value, released FAAs directly contribute to the basic taste of dry fermented meat products, and indirectly contribute to the development of their typical aroma since they are precursors of many volatile compounds [20]. However, an excessive amount of FAAs seems to be responsible for the onset of unpleasant sour and bitter taste [21]. Thus, FAAs play a key role in nutritional value, consumer acceptance, and sensory attributes of dry-cured fermented meat products [22]-[23]. The concentration of amino acids associated with sweet, bitter, acidic, and aged tastes has been calculated. In the text, the term “sweet” taste amino acids correspond to the sum of threonine, serine, proline, and alanine. The sum of valine, methionine, isoleucine, leucine, histidine, and arginine is associated with “bitter” taste. Acids taste amino acids are aspartic acid and glutamic acid and histidine. “Aged” taste has been calculated as a sum of aspartic acid, tyrosine, and lysine [24]. In samples prepared with the traditional salting method, active proteolysis was observed during meat maturation, leading to the gradual release of free amino acids, including isoleucine, leucine, histidine, and arginine, from muscle proteins. The data presented in the table show that the content of these amino acids increased over the course of maturation, indicating intensive protein degradation and enhanced enzymatic activity. In addition to their nutritional value, these amino acids are bitter-tasting; therefore, their increased levels may also affect the product's taste and sensory characteristics by contributing to a more pronounced flavor. In contrast, these amino acids were present in lower concentrations in the samples with added starter cultures. The use of *L. rhamnosus* MDC 2012 strain during pork meat maturation shortened the process by 3 days and increased the content of essential amino acids. This enhances the biological value of the processed pork, particularly through elevated levels of L-glutamic acid (from 2.93% to 4.00%), L-lysine (from 0.48% to 1.23%), L-serine (from 0.83% to 1.57%), and L-leucine (from 1.3% to 1.43%) [25]. Among the free amino acids released during maturation, L-glutamic acid (together with L-aspartic acid) is a major contributor to the umami taste, often

described as a “brothy» or «soupy” flavor that enhances sensory perception. The marked increase of L-glutamic acid in our results highlights the role of *L. rhamnosus* MDC 2012 in umami enhancement, representing an important novelty for meat quality and consumer acceptability. In addition, the elevated levels of L-lysine and L-leucine further improve the product’s biological value, while the identification of more than 21 proteases in the genome of *L. rhamnosus* MDC 2012 suggests their involvement in the observed quality improvement.

***L. rhamnosus* whole-genome sequencing:** The overview of the whole-genome sequences of *L. rhamnosus* MDC 2012 is presented in Table S1 of the supplementary materials. Currently, the genome comprises 40 contigs, totaling approximately 3,007,568 nucleotides. The chromosomal replication initiator protein DnaA gene CDS is depicted in Fig. S1 and is located in contig 3 of our sequence. The contig 3 probe, which is 13,320 nucleotides long and contains the DnaA gene CDS, exhibits 99.98% identity with the complete genome of *Lacticaseibacillus rhamnosus* strain NS2301G1 (GenBank: CP166134.1), which has 2,968,519 base pairs in its circular DNA. This information underpins our strain classification, supporting previous classifications based on 16S rDNA but providing more detailed genomic evidence.

It will be separately noted that the studied genome contains a 64,499 bp linear plasmid in contig 54 and a 3,560 bp circular plasmid in contig 48, with 2 and 255 copies, respectively (Table S1). Their potential roles in meat fermentation will be discussed separately.

Relevance of whole-genome sequences to the meat fermentation activity of Lacticaseibacillus rhamnosus: The annotated proteins with proteolytic activity are presented in Table 3.

Table 3. The annotated proteins of *Lacticaseibacillus rhamnosus* that exhibit proteolytic activity.

Protein	Contig	CDS	Chain
PII-type proteinase	51	15719-18823	+
PII-type proteinase	51	18862-19287	+
PII-type proteinase	51	19262-20602	+
PIII-type proteinase	51	20771-21706	-
sigma-W protease RasP	23	2040-3281	+
ATP-dependent protease ATPase subunit ClpY	23	197952-199061	-
ATP-dependent protease subunit HslV	23	199403-199927	-
Carboxy-terminal processing protease CtpA	23	214299-215756	-
ATP-dependent Clp protease	23	261665-262915	-
putative ATP-dependent Clp protease	2	119570-121720	+
ATP-dependent Clp protease	2	294340-294942	+
ATP-dependent Clp protease	24	19610-21727	-
Rhomboid protease GluP	24	135951-136436	+
ATP-dependent zinc metalloprotease FtsH	1	1040-1405	-
ATP-dependent zinc metalloprotease FtsH	1	1664-1924	-
ATP-dependent zinc metalloprotease FtsH	1	2508-2900	+
ATP-dependent zinc metalloprotease FtsH	1	2864-3169	+
ATP-dependent zinc metalloprotease FtsH	1	3166-4113	+
Putative cysteine protease YraA	3	384699-385205	+
ATP-dependent Clp protease proteolytic subunit	46	55211-55801	+
ATP-dependent Clp protease proteolytic subunit	47	7684-8100	+

The abundance of proteolytic enzymes is clear evidence of the excellent meat-fermenting activity of the studied LAB.

The search for bacteriocin genes, such as pln (pediocin-like bacteriocins), nson (nisin-like genes), and lcn (lactococcin), in the *L. rhamnosus* genome revealed only lactococcin metabolism genes, specifically lcnD_1 and lcnD_2, which encode Lactococcin A secretion proteins (LcnD) located on contig 28. Other lcn genes may be absent due to the incomplete nature of the genome. These findings suggest the potential of *L. rhamnosus* to produce prebiotics.

Table 4. Functional gene product groups identified in the *L. rhamnosus* MDC 2012 proteome related to probiotic characteristics

Protein	Contig	CDS	Chain
Biofilm regulatory protein A	3	118567-119661	-
Biofilm regulatory protein A	51	48257-49315	-
Biofilm regulatory protein A	2	117363-118256	-
Cell division protein FtsL	8	11850-12215	+
Cell division protein SepF	37	1396-1851	-
Cell division protein FtsZ	37	1799-2839 division	-
Cell division protein FtsZ	37	2836-3132	-
Cell division protein FtsA	37	3144-4022	-
Cell division protein FtsA	37	4016-4492	-
Cell division protein DivIB	37	4541-5464	-
Cell division protein FtsX	46	12011-12343	-
Cell division ATP-binding protein FtsE	46	13010-13348	-
Cell division ATP-binding protein FtsE	46	13010-13348	-
Cell division ATP-binding protein FtsE_2	46	19239-19925	+
Cell division protein FtsX	46	19915-20802	+
Cell division topological determinant MinJ	46	21073-22170	+
Cell division protein ZapA	12	38551-38796	-
Lipoteichoic acid synthase LtaS1_1	35	90755-91912	-
Lipoteichoic acid synthase LtaS1_2	40	2890-4983	-
33 kDa chaperonin HslO	1	4432-5316	+
10 kDa chaperonin GroS	51	50191-50472	+
60 kDa chaperonin GroL	51	50508-52142	+
Putative glycosyltransferase EpsH_2	53	73528-74232	+
Putative glycosyltransferase EpsH_3	2	170801-171214	+
Putative glycosyltransferase EpsH_1	3	113791-114210	-

The analysis of the plasmid-like structure in contig 54 revealed various metabolism-related proteins, including PTS transporters, kinases, hydrolases, transposases, restriction enzymes, DNA ligase, DNA polymerase, and others. Additionally, more than 40% of the proteins are hypothetical. These results indicate the metabolic potential of this relatively large plasmid.

The proteome of *L rhamnosus* 255-copy circular plasmid is presented in Table 5.

Table 5. The proteome of *L. rhamnosus* circular plasmid

Protein	Protein BLAST	% of Homology	Contig	CDS	Chain
Hypothetical protein	Lysozyme [Escherichia coli]	86.79%	48	142-462	+
Hypothetical protein	Glycoside hydrolase family protein, partial [Enterococcus faecium]	100.00%	48	459-617	+
Hypothetical protein	Endopeptidase Rz [Escherichia phage PhiR30_1]	98.50%	48	614-1015	+
Hypothetical protein	Serum resistance lipoprotein Bor [Escherichia coli]	98.97%	48	1106-1399	-
Putative protein YdfO	Head protein [Escherichia phage Lambda]	100.00%	48	1689-2099	-
Hypothetical protein	Protein YbcW [Escherichia coli]	98.53%	48	2385-2591	+

The analysis of the proteome of the 255-copy circular plasmid revealed five hypothetical proteins and one YdfO protein (containing the DUF1398 domain), which is associated with stress response mechanisms or involved in processes such as DNA repair, protein folding, or cellular stress regulation. Among the hypothetical proteins, AINLLPND_00809 is 100% analogous to a phage endolysin [Escherichia coli], AINLLPND_00810 is 100% identical to a glycoside hydrolase family protein [Escherichia coli and other bacteria], AINLLPND_00811 is 100% identical to an Rz-like spanin [Escherichia phage Lambda], AINLLPND_00812 is 100% identical to a serum resistance lipoprotein Bor [Bacteria] (which helps bacteria resist the host's immune system), and AINLLPND_00814 is 100% identical to YbcW [Bacteria] (proteins similar to YbcW are believed to be involved in stress response, transport, or regulatory processes, although their specific functions are not definitively assigned). Overall, this plasmid is likely to confer excellent probiotic characteristics to its host and could be very useful in meat fermentation processes.

CONCLUSION: The obtained data suggest that the application of starter cultures accelerates proteolysis and the transformation of nitrogenous compounds. In particular, the rapid decrease in creatinine observed in the presence of *Lactobacillus rhamnosus* MDC 2012 indicates its strong proteolytic potential, which may enhance the maturation rate and improve the flavor intensity of the final pork product.

The data show that the content of essential amino acids increased during maturation, indicating increased protein degradation and enhanced enzymatic activity. The use of *L. rhamnosus* MDC 2012 during pork meat maturation shortened the process by 3 days and increased the content of essential amino acids. The abundance of proteolytic enzymes in the genome of *L. rhamnosus* provides clear evidence of its excellent meat-fermenting activity.

The whole-genome sequence of *L. rhamnosus* MDC 2012 revealed excellent meat fermenting and probiotic characteristics of the studied LAB. The meat-fermenting activity is associated with a powerful proteolytic complex. Meanwhile, the probiotic properties are linked to various antibacterial agents, including gene products encoded on high-copy-number plasmids and bacteriocins such as lactococcin.

The results of this study highlight the potential of *L. rhamnosus* MDC 2012 as a functional microorganism that contributes to the nutritional and sensory quality of fermented meat products. The observed enhancement of amino acid composition and bioactive compounds is consistent with the general principles of functional foods described by Martirosyan and Singh (2015), who emphasized the probiotic nature of fermented foods such as sauerkraut and their health-promoting properties.

Similarly, the demonstrated probiotic activities

of *Lacticaseibacillus paracasei* 327 in improving intestinal health further support the functional food potential of lactic acid bacteria strains, including *L. rhamnosus*, in various food matrices. These findings confirm that fermented meat products enriched with such probiotic cultures can be considered as a promising category of functional foods [26]. The present study demonstrates that *L. rhamnosus* MDC 2012 not only accelerates proteolysis and amino acid release but also contributes to the generation of bioactive peptides with potential health benefits, thus linking the findings to the principles of Functional Food Science. These peptides, together with probiotic metabolites, may exert antioxidative, antimicrobial, and immunomodulatory effects, aligning with current concepts of bioactive compounds in functional foods. Similar associations between proteolytic activity, biofilm formation, and probiotic relevance in fermented meat systems have recently been reported by Manvelyan et al. Furthermore, the amino acid composition enhancement observed in this study is consistent with that reported by Grigoryan et al., who demonstrated that amino acid enrichment improves the nutritional quality and safety of meat-based products. Related research has also confirmed that amino acid metabolism and bioactive compound synthesis are essential indicators of meat safety and health-promoting properties. Moreover, recent studies highlight the nutritional role of animal-derived proteins in supporting human health and development. Taken together, these results confirm that fermented pork products containing *L. rhamnosus* MDC 2012 can be considered not only high-quality traditional foods, but also functional foods enriched with bioactive components that may exert positive effects on human health [27-30].

Abbreviations: Bas: Biogenic Amines, FAAs: Free Amino Acids, HAAs: Heterocyclic Aromatic Amines, LAB: Lactic Acid Bacteria, MDC: Microbial Depository Center, NGS: Next Generation Sequencing, WDCM: World Data Center for Microorganisms.

Data Availability Statement: The supplementary materials (Supplementary materialN_L_ rhamnosus_Hasmik.docx) and genome data files (FastQ_pass_merged1246_ End.prokka.gbk and FastQ_pass_merged1246_ End.prokka.gff) for this article are available at are deposited in Mendeley Data at https://data.mendeley.com/datasets/z6g7hw2sfh/3 under the *"Lacticaseibacillus rhamnosus* MDC 2012 whole-genome" project.

Competing Interests: The authors have no financial interests or conflicts of interest.

Authors' Contributions: H. G. designed sample preparation and experimental procedures. F. T., K. K., and A. P. conducted microbiological studies. T. S. and L. K. performed DNA extraction, electrophoresis, sequencing analysis, and preliminary data interpretation. A. H. carried out whole-genome sequencing, and G. M. and H. G. prepared the manuscript, figures, and references.

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