applied sciences

MDPI

Article

Listeria Monocytogenes in Soft Spreadable Salami: Study of the Pathogen Behavior and Growth Prediction During Manufacturing Process and Shelf Life

Raffaella Branciari [1], Roberta Ortenzi [2], Rossana Roila [1,*], Dino Miraglia [1], David Ranucci [1,*] and Andrea Valiani [2]

[1] Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy; raffaella.branciari@unipg.it (R.B.); dino.miraglia@unipg.it (D.M.)

[2] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Via G. Salvemini 1, 06126 Perugia, Italy; r.ortenzi@izsum.it (R.O.); a.valiani@izsum.it (A.V.)

* Correspondence: rossana.roila@unipg.it (R.R.); david.ranucci@unipg.it (D.R.)

Received: 10 June 2020; Accepted: 25 June 2020; Published: 27 June 2020

Abstract: Recently, particular attention has been addressed to the control of *Listeria monocytogenes* in ready-to-eat meat products, such as fermented salami, as a consequence of several listeriosis outbreaks associated with the consumption of these types of products. A short-ripened spreadable salami, typically produced in the Umbria region (Central Italy), was challenged with *L. monocytogenes* aiming to evaluate the pathogen's growth dynamics and to define its growth potential during processing and storage time. The pathogen counts were stable in the inoculum level (2 Log CFU/g) during the production process and up to 30 days of storage time, decreasing thereafter. The growth potentials registered for process phase and storage time were 0.40 and −1.28, showing that the application of the hurdles technology principle successfully creates an unfavorable environment for *L. monocytogenes* growth.

Keywords: challenge test; ready-to-eat meat product; growth potential; short-ripened salami; safety of food of animal origin

1. Introduction

Listeria monocytogenes is an ubiquitous Gram-positive bacterium able to contaminate a wide variety of foodstuffs; particularly, it is considered a relevant foodborne pathogen commonly associated with ready-to-eat (RTE) products [1,2]. The microorganism is responsible for numerous outbreaks of listeriosis worldwide [3–6] with high mortality rates in specific population categories such as immunocompromised subjects, elderly and pregnant women [7–9]. *L. monocytogenes* is also considered a real concern in the meat industry. Once the microorganism is introduced into a meat processing plant through contaminated unprocessed raw materials, and due to its distinct characteristics of resistance to environmental conditions, it can survive through the manufacturing process [10–12]. Furthermore *L. monocytogenes* is able to adhere to surfaces in the food-processing environments and, once attached, it may produce biofilms that are resistant to disinfection and from which cells can become detached and constantly contaminate food products [13,14]. As a consequence, in spite of food producers' efforts, *L. monocytogenes* may not be completely eliminated during the production of dry-fermented meat products [15], suggesting that further measures must be taken to avoid the growth of this pathogen [16,17]. In the literature, it is reported that *L. monocytogenes* growth during fermentation and drying of meat products can decrease due to the combined action of several hurdles

such as the reduction in water activity (a_w) and pH or the employ of additives, such as nitrite and nitrate salts [18,19]. However, in some cases, the physicochemical parameters of fermented salami can be permissive for *Listeria* growth [20,21], particularly for those products with short ripening [22]. In Central Italy, there are several RTE spreadable fermented meat products with short ripening times and high fat contents [23–26]. The characteristics of these RTE salami products together with the short maturation could increase the chance that *L. monocytogenes* survives and proliferates during the manufacturing process. For RTE foods that are able to support the growth of *L. monocytogenes,* food business operators (FBO) can benefit from scientific studies in order to demonstrate that the product complies with the regulation's criteria throughout the shelf-life [27–29]. Nevertheless, few studies and practices are found in the literature that could be applied by FBO to their own products, especially for artisanal and niche ones [30]. The aim of the present investigation was to evaluate the growth dynamics of *L. monocytogenes* and to define its growth potential during the processing and storage time of an artisanal spreadable "Monti Sibillini" salami manufactured by a producer in the Umbria region (Central Italy), and characterized by a short ripening time and a soft consistency. Furthermore, this study also aimed to propose a valuable scientific tool enabling food manufacturers who produce salami with similar characteristics to prove whether their product is supportive of *L. monocytogenes* growth, according to EC Regulation No. 2073/2005 [28].

2. Materials and Methods

2.1. Bacterial Cultures and Inoculum Preparation

A multi-strain mix of *L. monocytogenes* was used in the study to carry out a challenge test. The mix consisted of an authenticated reference strain (WDCM 00021) and two strains from Istituto Zooprofilattico Sperimentale dell'Umbria and Marche "Togo Rosati" (IZSUM) collection isolated from food matrices (Lm15011/14 from salami and Lm36206/14 from dry sausage); each strain was lyophilized and kept at +4 °C in the IZSUM collection. The inoculum to be used in the challenge study of salami was prepared according to the guidelines of the European Union Reference Laboratory for *Listeria monocytogenes* [31]. Briefly, the bacterial cultures used in the study were regenerated into Brain Heart Infusion (BHI; Oxoid, Basingstoke, UK) by incubation at 37 °C for 24 h. Aliquots of each activated culture (0.1 ml) were transferred into tubes containing BHI and incubated for 72 h at 10 ± 1 °C, a temperature close to the storage condition of the product. At the end of the incubation time, the cultures were equally combined to form the multi-strain cocktail, which was subsequently centrifuged at 2178 g at 10 °C for 5 min, the supernatant was discarded and the pellet resuspended in 10 mL of sterile physiological solution. Counts were performed by serial decimal dilution and inoculation in Agar Listeria Ottaviani Agosti (ALOA Selective Supplement, ALOA Enrichment Supplement; Biolife, Italy), incubated at 37 °C for 24−48 h and the multi-strain suspension was diluted with sterile physiological solution to obtain an inoculum able to determine a final concentration in meat batter of approximately 2 Log Colony Forming Units (CFU)/g.

Generally, a microbiological challenge test assessing the *Listeria monocytogenes* growth potential (δ) is performed only on final meat products, starting at the day of product contamination and finishing at the end of the shelf life [31]. In this study, the contamination was performed at the beginning of the production process (into meat batter), taking into account the most probable conditions of natural contamination of the product and the specific packaging choices of the manufacturer (the salami is commercialized whole and under vacuum). Therefore, the evaluation of the pathogen's behavior, as well as all the other analytical determinations, were performed during both the production process and the shelf life.

2.2. Salami Production

The soft spreadable salami investigated here is a typical product of the Umbria region, in Central Italy. Its soft consistency is attributable to the high level of fat, the fine mincing of meat and the specific

processing techniques. Meat batter was prepared using the following swine cuts: shoulder 30%, jowl and belly (70%). Moreover, salt 2.7%, ground black pepper 0.4%, ground garlic 0.1%, white wine (10 mL/kg), L-ascorbic acid (E301), potassium nitrate (E252) and sodium nitrate (E250) were also added in compliance with EC regulation N° 1333/2008 including subsequent amendments and additions [32]; no starter cultures were employed. For each batch, a total of 15 kg of meat batter was processed. The meat batter was minced twice with a 2.5 mm plate and then stuffed into casings of approximately 500 g each. Subsequently, the products underwent a drying process in a controlled environment for about 5 days, with decreasing temperature (T) and relative humidity (RH), as detailed below: T 24 °C RH 65% for 9 h, T 23 °C RH 65% for 3 h, T 22 °C RH 65% for 24 h, T 21 °C RH 60% for 20 h, T 21 °C RH 55% for 18 h, T 20 °C RH 50% for 14 h, T 19 °C and RH 45% for 28 h. The ripening phase took place in a controlled environment for another 5 days, with a T of 14–16 °C and an RH around 70%. Each salami was vacuum packaged and stored at +4 °C for 60 days.

2.3. Preparation of Samples

To conduct the challenge test, three batches of salami were produced, each batch consisted of 3 experimental groups of 6 products sized 500 g.

The first group (LM) was produced at the pilot plant of the IZSUM by experimentally contaminating the meat batter with a suspension of multi-strain mix of *L. monocytogenes* at a final concentration of approximatively 2 Log CFU/g. These sample units were used to evaluate the behavior of *L. monocytogenes* during salami production and ripening.

The second group (CTR) was produced at the pilot plant of the IZSUM by adding an amount of sterile saline solution, instead of the contaminating suspension, equivalent to the inoculum (50ml). These sample units were employed to determine the physicochemical characteristics of salami (considering also the potential influences of saline solution), to detect and/or enumerate the eventual *L. monocytogenes* occurring naturally in the products and to ensure that the same ripening condition and product evolution occurred in both the manufacturing process and the IZSUM. A third group (CTRFBO) was produced and ripened by the manufacturer in order to assure that the products that are routinely produced by the processor are equivalent to those submitted to experimental contamination.

2.4. Storage Conditions and Sampling

LM and CTR samples were ripened at the IZSUM according to the maturation profile (time/T/RH) applied by the producer and subsequently packaged under vacuum.

The shelf life defined by the manufacturer for the salami was equivalent to 60 days (starting from the end of ripening phase) stored at +4 °C therefore according to the EURL reference document [31], after ripening, the products were stored at +8 °C for 7 days and at +12 °C for the remaining storage time (53 days).

Microbiological and physicochemical analyses were performed at 0 (day of production), 1, 2, 5, 10 (maturation time), 40 and 70 days (storage time). All analyses were carried out on two replicates of salami and three replicates for each batch (N = 3 batch; n = 3 replicates per sampling time); at time 0 the sample was constituted by meat batter (approximately 100g), while at the following time points the sampling unit was a whole salami unpacked up until day 10, vacuum packed at 40 and 70 days.

2.5. Microbial Analysis

2.5.1. Enumeration and Isolation of Viable Lactic Acid Bacteria

The enumeration of lactic acid bacteria (LAB) was conducted at 0, 1, 2, 5 and 10 days of maturation and at 40 and 70 days of storage time, in CTR samples of each batch according to the method reported by Ortenzi et al. [4]. The analyses were conducted using the Tempo®automated system (Tempo LAB®, bioMérieux, Mercy Etoile, France) that has been proved to achieve performance levels similar to the standard NF ISO 15214, 1998 [33,34]. Counting results were reported in terms of Log CFU/ g.

2.5.2. Detection and Enumeration of Listeria Monocytogenes

The detection and enumeration of *L. monocytogenes* were conducted following the previously reported sampling times in all sample units of each batch according to Annex I of Regulation No. 2073/2005 [28]. The validated method AFNOR BIO 12/11–03/04 [35] for the detection of *Listeria monocytogenes* in human food products was employed. Briefly, a pre-enrichment was performed in half-Fraser broth (Biolife, Italy), incubated for 24 h at 30±1 °C and then sub-cultured in Fraser broth (Biolife, Italy) and incubated for 24 h at 37±1 °C. Subsequently the VIDAS LMO2 test (VIDAS®, bioMérieux, Mercy Etoile, France) was performed. Samples found to be positive to the mentioned test are confirmed through isolation on ALOA growth medium. The limit of detection of the method is of 0.4 CFU in 25 g. For bacterial enumeration, the EN ISO 11290-2 [36] reference method was used with a limit of detection of 10 CFU/g. Counting results were reported in terms of Log CFU/g.

2.6. Physicochemical Determination

The physicochemical determinations were carried out at 0, 1, 2, 5 and 10 days of ripening and at 40 and 70 days during storage time in the sample units of each batch belonging to the CTR groups.

The pH measurements were performed through a puncture electrode probe connected to a portable pH meter (Mettler Toledo Inc., Columbus, OH, USA). The salt (NaCl) content was determined by the Volhard method according to AOAC (method n 935.43) [37]. Water activity (aw) was measured at 25 °C with the a_w recorder AquaLab, series 3, Model TE (Decagon Devices Inc., Pullman, WA, USA).

2.7. Statistical Analysis

Bacterial counts were converted to Log CFU per g or mL. The individual means and standard deviations of the microbiological and physicochemical results were determined on the basis of the average of two samples. The data were statistically analyzed using SAS version 2001 (SAS institute inc., Cary, NC, USA).

Differences between mean values were detected through the Post-hoc Tukey's test and evaluations were based on a confidence interval of 95%. The growth potential (δ) of *L. monocytogenes* is estimated as the difference between the median of results at the end of the challenge test and the median of results at the beginning of the challenge test, in three replicates, for three batches [31].

During the production process and shelf life of the product, the fitting of *L. monocytogenes* growth curves was performed using the free software program DMFit (https://www.combase.cc/index.php/en/DMFit) to measure growth parameters such as maximum specific growth rate (μmax, 1/h) and Lag time (λ, 1/h) using the model of Baranyi and Roberts [38].

3. Results and Discussion

In total, 54 salami (3 batches/3 groups/6 salamis) of 500 g each were produced. Three samples from each salami were submitted to analytical determinations during the testing period. Table 1 shows the results of the physicochemical determinations and LAB enumeration performed on CTR and CTRFBO salamis during ripening and storage. No significant differences ($p < 0.05$) were detected among the two groups, therefore the discussion took into account the characteristics of the spreadable salami produced by the processor.

The results highlight that the acidification process had taken place since the early stages of ripening, reaching the lowest value at day 10 (5.27). During the storage time the pH tended to show a slight increase reaching a final value (day 70) of 5.44, as reported elsewhere in different types of salami [39].

A reduction in a_w values for salami occurred gradually and constantly throughout maturation and storage, from an initial value of 0.961 for the batter to a final value (day 70) of 0.931 (Table 1). The NaCl value, as expected, increased with ripening and storage time from an average value of 2.47% at day 0 to a final mean value (day 70) of 3.39% (Table 1).

Table 1. Physicochemical and microbial characteristics of salami during ripening and storage of spreadable salami. Data represent the average values of three replicate samples for three batches.

		Production Process					**Storage**		**SEM**	***P value***		
										S	**Y**	**SxT**
Days		0	1	2	5	10	40	70				
pH	CTRFBO	5.82w	5.76w	5.31v	5.35v	5.27v	5.39v	5.44v	0.066	0.597	<0.001	0.968
	CTR	5.82x	5.74x	5.39vw	5.30v	5.28vw	5.43vw	5.50w				
aw	CTRFBO	0.961y	0.961y	0.959y	0.949x	0.948x	0.938w	0.931v	0.001	0.454	<0.001	0.420
	CTR	0.961y	0.961y	0.959y	0.951x	0.946x	0.938w	0.934v				
NaCl	CTRFBO	2.47v	2.84vw	2.95wx	3.09xy	3.23xy	3.23xy	3.39y	0.084	0.899	<0.001	0.981
	CTR	2.47v	2.76w	2.97wx	3.15xy	3.20xy	3.29xy	3.41y				
LAB (Log CFU/g)	CTRFBO	4.06v	6.56w	7.80x	8.97y	8.94y	8.57y	7.92x	0.103	0.857	<0.001	0.995
	CTR	4.06v	6.53w	7.72x	8.94x	8.91x	8.59y	7.99x				

Different letters in the same row (v, w, x, y, z) indicate differences between mean values during sampling times ($p \leq 0.05$). No differences were registered within the same column for each parameter considered. CTR, produced by the food business operator and ripened/stored at the Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"; CTRFBO, samples produced and ripened/stored by the manufacturer in order to compare the products submitted to challenge testing to the products that are routinely produced by the processor. SEM, standard error mean.

The physicochemical properties of the salami investigated in this study are in agreement with those obtained by other authors in similar products [23,24,40,41], although a_w values registered were higher than the threshold of 0.90 proposed for the microbial stability in order to prolong the shelf life of salami [19].

To evaluate the background microbiota, the lactic acid flora during the ripening and shelf life time was investigated (Table 1). The results of the behavior of LAB during ripening and storage showed no difference among batches.

A significant growth ($p < 0.05$) of LAB was observed until the end of the manufacturing process with counts reaching 8.94 Log CFU/g at day 10 (LAB increase in 4 Log CFU/g from day 0). At the end of storage time (70 days) the LAB decreased to a value of 7.92 Log CFU/g (Table 1).

The comparison of the results obtained from the analysis of the three batches showed no intrabatch nor interbatch variability of the physicochemical properties or of the background microbiota, confirming that the production process of the studied salami is fully standardized, differing from what was reported by other studies of analogous products [30]. This outcome also confirms the importance of considering multiple batches and various replicates per batch in order to establish the inherent variability in physicochemical characteristics and growth potential linked to the product and its processing/storage conditions, as recommended by Annex II on challenge testing of EC Regulation 2073/2005 [28].

As mentioned above, in the present study, the contamination was performed on meat dough, and the evaluation on the pathogen evolution and the definition of the growth potential (δ) was performed during both the production process and the shelf life.

The behavior of *L. monocytogenes* during spreadable salami ripening and storage time, considering the three batches of LM samples, is shown in Figure 1; the pathogen was undetectable in control samples. *L. monocytogenes* artificially inoculated in salami was able to survive throughout the ripening time (2.43 ± 0.19 Log CFU/g at 10 days) while a decrease in the pathogen concentration was observed during storage (1.07 ± 0.11 Log CFU/g at 70 days; Figure 1). At the end of the storage period (day 70) for batch 1 the *L. monocytogenes* was undetectable (<10 CFU/g) (data not shown).

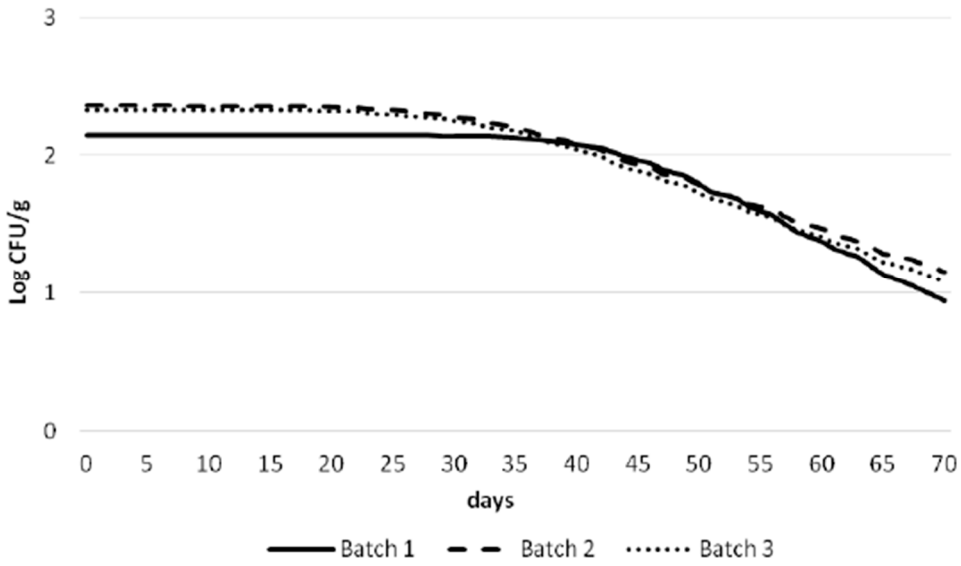

Figure 1. Growth profile of *Listeria monocytogenes* (Log CFU/g) during spreadable salami ripening and storage (data represent the average values ± standard deviation of three replicates samples for three LM batches). Different superscript letters (a, b, c) indicate differences between *L. monocytogenes* counts ($p < 0.05$).

Concerning δ definition, as reported in Table 2A, at day 0 (beginning of production process) the median concentrations of *L. monocytogenes* were 2.15 Log CFU/g, 2.23 Log CFU/g and 2.10 Log

CFU/g for batch 1, 2 and 3, respectively. The calculated growth potential of *L. monocytogenes* for the targeted spreadable salami was 0.13 Log CFU /g in batch 1 and 0.40 Log CFU /g in batch 2 and batch 3. The highest δ value obtained is retained among all tested batches, therefore, for the production process the δ is 0.40 Log CFU/g [31]. The calculated δ is ≤ 0.5 Log CFU/g, therefore, according to reference guidelines, it is assumed that during this time frame the salami is not able to support the growth of *L. monocytogenes* [31]. This evidence corroborates the results previously reported for the pathogen behavior (Figure 1); indeed, no increase in *L. monocytogenes* loads was observed at any time during the process, whereas, a decreasing trend of the pathogen concentration was detected during the storage time (from day 10 to day 70).

Table 2. Calculation of growth potential of *Listeria monocytogenes* during ripening (**A**) and storage period (**B**) of experimentally contaminated spreadable salami.

Batch	Day	Concentration (Log CFU/g)	SD (Standard Deviation)	Median	Growth Potential (δ) per Batch	Highest δ among the 3 Batches
A						
1	0	2.15 2.18 2.15	0.02	2.15	2.28−2.15 = 0.13	
	10	2.3 2.28 2.04	0.14	2.28		
2	0	2.23 2.3 2.11	0.09	2.23	2.63−2.23 = 0.40	0.40
	10	2.08 2.63 3.04	0.48	2.63		
3	0	2.10 2.13 2.05	0.04	2.10	2.50−2.10 = 0.40	
	10	2.50 2.40 2.56	0.08	2.50		
B						
1	0	2.3 2.28 2.04	0.14	2.28	<1−2.28 = >−1.28	
	70	<1 <1 <1	0	<1		
2	0	2.08 2.63 3.04	0.48	2.63	1.00−2.63 = −1.63	−1.28
	70	1.48 1 1	0.28	1		
3	0	2.50 2.40 2.56	0.08	2.50	1.10−2.50 = −1.28	
	70	1.17 1.00 1.10	0.09	1.10		

At day 0 of storage (that corresponds to the sampling day 10) the registered median concentrations of *L. monocytogenes* were 2.28 Log CFU /g for batch 1, 2.63 Log CFU /g for batch 2 and 2.50 Log CFU /g for batch 3 (Table 2B). The growth potential of *L. monocytogenes* was −1.28 Log CFU /g for batch 1 and 3 and −1.63 Log CFU /g for batch 2, therefore, concerning the storage time of spreadable salami, the δ is −1.28 Log CFU/g. As reported for the production process, in the conservation time the calculated δ is also lower than 0.5 Log CFU/g, confirming that the product is unsupportive of *Listeria* growth [31]. Furthermore, in this phase, not only is the calculated δ below the limit set by the reference document, but it is also represented by a negative value (< 0), suggesting, therefore, that during the conservation time the hurdles applied to the spreadable salami are able to exert their action against the growth of *Listeria*, decreasing the pathogen concentration (Figure 1 and Table 2B).

The growth curves of *L. monocytogenes* in experimentally produced spreadable salami contaminated at the beginning of the production process and stored according to the abovementioned conditions were obtained by fitting the observed growth data using the DMFit program (Figure 2).

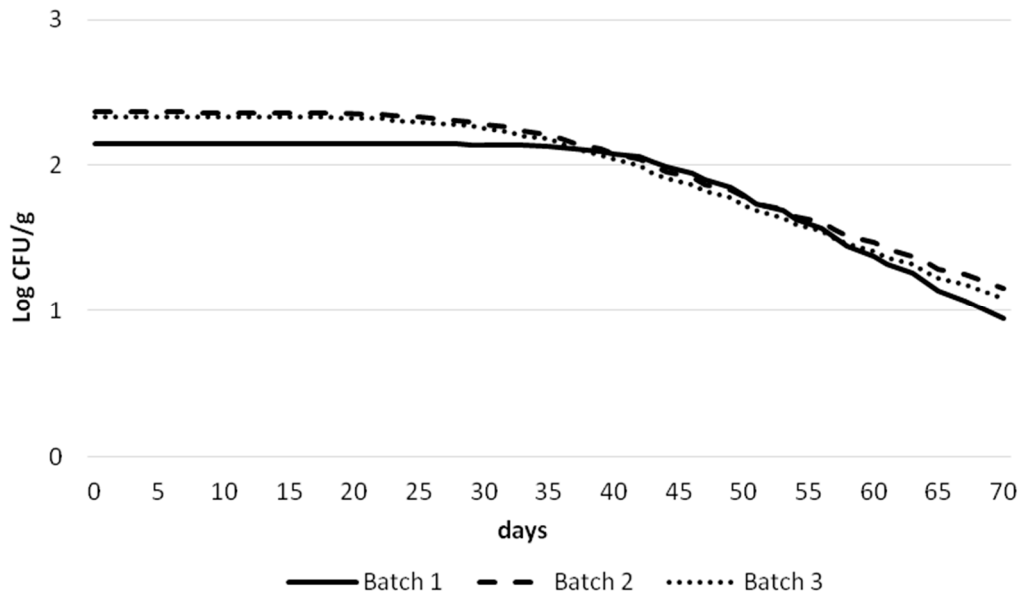

Figure 2. Growth curves of *L. monocytogenes* fitted by the DMFit program according to the Baranyi and Roberts model [38] for three different batches of spreadable salami experimentally contaminated.

According to the Combase Predictor tool (part of the Modelling Toolbox, www.combase.cc) [42] in culture media with equivalent growth conditions of salami, a maximum *L. monocytogenes* population of 8.51 log CFU/g was reached in about 625 h (26 days, data not shown). However, the fitted growth curves show that the pathogen concentration reached a maximum level of 2.43 log CFU/g and then the growth was suppressed (Figure 2), corroborating the results reported above concerning the definition of *Listeria* growth potential. The DMFit program was also used to calculate growth parameters for each condition tested (Table 3). The maximum specific growth rates (μmax, 1/h) ranged from −0.0019 to −0.0013, while the latency phase (Lag) ranged from 776 to 1032 h. The lower asymptote was not reached at the end of the considered shelf life, therefore the final values for the three batches were not determined.

The results of the fitting of observed growth data corroborate the outcomes of the challenge test. For each of the three growth curves, in fact, the value of μ_{max} was negative, confirming that the growth of the targeted pathogen was suppressed in the food environment considered.

As shown in Table 3, the latency phase (Lag) of the three fitted curves ranges from a minimum value of 32 days (780 h) to a maximum value of 42 days (1032 h) for batch 2 and 1, respectively. The values registered in this study are remarkably higher than the value obtained through the growth

prediction performed with the Combase Predictor tool [42] that was of 0.72 days (17.3 h, data not shown). As reported in the literature, the duration of the latency phase is characterized by inherent variability and by partially defined physiological and molecular processes [43], however, recent findings suggest that changes in the lag-time in order to develop tolerance can be considered the first change made by bacteria in response to stressor factors [44]. The optimization and elongation of Lag may therefore represent a strategy adopted by bacterial communities to tolerate environmental stress [43–45], that may correspond, in this study, to the hurdles present in the food matrix.

Table 3. Output parameters estimated by the DMFit program for each growth curve in three batches of experimentally produced spreadable salami.

Batch	μ_{max} (Log CFU/h)	Lag (h)	SE	R^2
Batch 1	-0.0019	1032	0.25	0.743
Batch 2	-0.0013	780	0.22	0.792
Batch 3	-0.0014	776	0.18	0.864

μ_{max} = specific maximum growth rate; Lag = lag phase; SE= standard error of fitting; R^2 = adjusted R-square statistics of the fitting.

These results demonstrate the inhibitory effect of the process and the product characteristics on *L. monocytogenes* growth, even though the studied spreadable salami showed a pH and a_w values compatible with the growth of the microorganism [28]. In fact, similarly to what has been reported by other authors, the physicochemical properties of spreadable salami are not sufficient to justify the pathogen behavior [41], indeed, it has already been highlighted that *L. monocytogenes* is able to survive in environments with pH and a_w values lower than those found in this study [46].

The factors that could affect the behavior of *L. monocytogenes* in RTE meat products are numerous, such as reductions in pH [47] and a_w [48], the use of additives [49], and the presence of indigenous microflora [50]. The presence of NaCl is considered a method to control the pathogen growth, however, the concentration used in the present experiment is not demonstrated to limit *L. monocytogenes* growth [51]. Similarly, the temperature applied during the ripening phase and the storage period could not have negatively influenced the growth capacity of *L. monocytogenes* since the strains used for experimental contamination had undergone a process of thermal adaptation. The main hurdle in *L. monocytogenes* growth, as shown in the literature, is probably represented by lactic acid flora [50]. Several studies have shown, in fact, that the presence of LAB in foods can hinder the growth of pathogens by means of competition for nutrients, antimicrobial metabolite production and more generally through microbial antagonism [52,53].

Specifically, as reported in the literature, the reduction in *L. monocytogenes* count in some foodstuffs may have been attributable to the inhibitory effects of various metabolic products of LAB such as carbon dioxide, short-chain organic acids, hydrogen peroxide, diacetyl, and antimicrobial peptides [54].

The inhibitory activity of LAB towards sensitive food spoilage or pathogenic bacteria, such as *L. monocytogenes*, has been reported for various food products [52,55,56]. Particularly, Al-Zeyara et al. [57] and Cornu et al. [58] state that the lactic flora present in fermented products, such as salami, exerts a remarkable inhibitory effect on *L. monocytogenes* growth especially when the aforementioned microflora is present in concentrations higher than 4.5 Log CFU/g by creating an unfavorable environmental condition for the pathogen.

Studies of the LAB population of spreadable salami produced in Central Italy revealed that the most frequently detected lactic species were *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Pediococcus pentosaceus* [25,40,59,60].

Some authors claim that the anti-listerial potential of bacteriocinogenic *L. sakei* and *L. curvatus* represents an efficient tool to counteract the development of pathogens such as *L. monocytogenes* in food, even at low temperatures [61–64].

Furthermore, evidences have also shown that non bacteriocin-producing strains of *L. sakei* are able to retard spoilage and to reduce the number of pathogens by developing dominant populations in vacuum packaged fresh meats [65]. The inhibitory potential of non bacteriocinogenic *L. sakei* strains might be attributable to the high specialization of the ecological niche represented by meat products, enabling this microorganism to be an efficient competitor for nutrients and to have a better colonizing capability [66].

Furthermore, some *L. plantarum* strains have been characterized as bacteriocin producers [67,68], highlighting their potential application as bio-preservatives in meat and meat products [69]. Indeed, some studies have suggested that certain strains of *L. plantarum* are able to produce class IIb bacteriocins with synergistic antibacterial effects [70]. Barbosa et al. [69] have also highlighted the remarkable antilisterial activity of *L. plantarum* isolated from a fermented salami and able to produce a two-peptide lantibiotic plantaricin.

Similarly to what was previously mentioned for *L. sakei* and *L. plantarum*, it has been reported in literature that *P. pentosaceus* effectively inhibits the growth of *L. monocytogenes* in pork fermented sausage, mainly due to the production of a non-lantibiotic class IIa bacteriocin [71]. It is likely that the inhibition of *L. monocytogenes* in the targeted spreadable salami was determined by the combination of the physicochemical parameters associated with the inhibitory effect of the lactic flora exerted mainly through the production of antimicrobial peptides, as reported by others authors [16,39,57]. Furthermore, it has been reported elsewhere that in presence of nitrate and nitrite additives, the fermentation process is able to limit and even suppress the growth of *L. monocytogenes* in fermented sausages [18]. In this study, the food matrix was challenged with *Listeria monocytohenes* during both ripening and storage by performing the contamination on meat batter at the beginning of the production process. This innovative approach enabled the thorough assessment of pathogen behavior in this peculiar meat production compared to challenge study only during shelf life [20,39]. Finally, the results of the present study are in agreement with the hurdle technology concept [72,73], which demonstrates that the combination and interaction of all the major preservative factors for foods are pivotal for optimal food preservation ensuring food stability and safety.

4. Conclusions

The dry-fermented process of salami, characterized by a short ripening time, could be a source of risk for consumers caused by *Listeria monocytogenes*. A useful strategy aiming to limit the presence and the growth of such pathogen is to favor the development of the native lactic flora that, in combination with other preservative factors such as additives, can act as a hurdle against *Listeria* creating an unfavorable environment for its survival.

Author Contributions: Conceptualization, A.V. and R.O.; methodology, R.O., R.B., R.R., A.V.; validation, R.B., R.O., and A.V.; formal analysis, R.O., R.R.; resources, A.V.; data curation, R.B, D.M.; writing—review and editing, R.B., R.R., D.R.; visualization, D.M.; supervision, R.B., A.V. D.R.; project administration, A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mamber, S.; Mohr, T.; Leathers, C.; Mbandi, E.; Bronstein, P.A.; Barlow, K.; Silverman, M.; Aston, C.; Izsak, Y.; Saini, N. Occurrence of *Listeria monocytogenes* in Ready-to-eat Meat and Poultry Product Verification Testing Samples from United States Department of Agriculture-regulated Producing Establishments, 2005–2017. *J. Food Prot.* **2020**. [CrossRef] [PubMed]
2. Pérez-Rodríguez, F.; Carrasco, E.; Bover-Cid, S.; Jofré, A.; Valero, A. Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: Activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage. *EFSA Support. Publ.* **2017**, *14*, 1252E. [CrossRef]

3. European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J.* **2018**, *16*, e05500.
4. Ortenzi, R.; Branciari, R.; Primavilla, S.; Ranucci, D.; Valiani, A. Behaviour of *Listeria monocytogenes* in artisanal raw milk Pecorino Umbro cheese: A microbiological challenge test. *Ital. J. Food Saf.* **2015**, *4*, 5370. [CrossRef] [PubMed]
5. Desai, A.N.; Anyoha, A.; Madoff, L.C.; Lassmann, B. Changing epidemiology of *Listeria monocytogenes* outbreaks, sporadic cases, and recalls globally: A review of ProMED reports from 1996 to 2018. *Int. J. Infect. Dis.* **2019**, *84*, 48–53. [CrossRef]
6. Smith, A.M.; Tau, N.P.; Smouse, S.L.; Allam, M.; Ismail, A.; Ramalwa, N.R.; Disenyeng, B.; Ngomane, M.; Thomas, J.; Thomas, J. Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: Laboratory activities and experiences associated with whole-genome sequencing analysis of isolates. *Foodborne Pathog. Dis.* **2019**, *16*, 524–530. [CrossRef]
7. Buchanan, R.L.; Gorris, L.G.; Hayman, M.M.; Jackson, T.C.; Whiting, R.C. A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control.* **2017**, *75*, 1–13. [CrossRef]
8. 8. European Food Safety Authority and European Panel on Biological Hazards (BIOHAZ). *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA J.* **2018**, *16*, e05134.
9. Simon, K.; Simon, V.; Rosenzweig, R.; Barroso, R.; Gillmor-Kahn, M. Listeria, Then and Now: A Call to Reevaluate Patient Teaching Based on Analysis of US Federal Databases, 1998–2016. *J. Midwifery Wom. Heal.* **2018**, *63*, 301–308. [CrossRef]
10. Al-Nabulsi, A.A.; Osaili, T.M.; Shaker, R.R.; Olaimat, A.N.; Jaradat, Z.W.; Elabedeen, N.A.Z.; Holley, R.A. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of *Listeria monocytogenes*. *Food Microbiol.* **2015**, *46*, 154–160. [CrossRef]
11. Bucur, F.I.; Grigore-Gurgu, L.; Crauwels, P.; Riedel, C.U.; Nicolau, A.I. Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. *Front. Microbiol.* **2018**, *9*, 2700. [CrossRef] [PubMed]
12. Mataragas, M.; Rantsiou, K.; Alessandria, V.; Cocolin, L. Estimating the non-thermal inactivation of *Listeria monocytogenes* in fermented sausages relative to temperature, pH and water activity. *Meat Sci.* **2015**, *100*, 171–178. [CrossRef] [PubMed]
13. Gómez, D.; Iguácel, L.P.; Rota, M.; Carramiñana, J.J.; Ariño, A.; Yangüela, J. Occurrence of *Listeria monocytogenes* in ready-to-eat meat products and meat processing plants in Spain. *Foods* **2015**, *4*, 271–282. [CrossRef] [PubMed]
14. Colagiorgi, A.; Bruini, I.; Di Ciccio, P.A.; Zanardi, E.; Ghidini, S.; Ianieri, A. *Listeria monocytogenes* biofilms in the wonderland of food industry. *Pathogens* **2017**, *6*, 41. [CrossRef] [PubMed]
15. Lindqvist, R.; Lindblad, M. Inactivation of Escherichia coli, *Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. *Int. J. Food Microbiol.* **2009**, *129*, 59–67. [CrossRef]
16. Mataragas, M.; Bellio, A.; Rovetto, F.; Astegiano, S.; Decastelli, L.; Cocolin, L. Risk-based control of food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* in the Italian fermented sausages Cacciatore and Felino. *Meat Sci.* **2015**, *103*, 39–45. [CrossRef]
17. Nyhan, L.; Begley, M.; Mutel, A.; Qu, Y.; Johnson, N.; Callanan, M. Predicting the combinatorial effects of water activity, pH and organic acids on Listeria growth in media and complex food matrices. *Food Microbiol.* **2018**, *74*, 75–85. [CrossRef]
18. Christieans, S.; Picgirard, L.; Parafita, E.; Lebert, A.; Gregori, T. Impact of reducing nitrate/nitrite levels on the behavior of *Salmonella typhimurium* and *Listeria monocytogenes* in French dry fermented sausages. *Meat Sci.* **2018**, *137*, 160–167. [CrossRef]
19. Meloni, D. Presence of *Listeria monocytogenes* in Mediterranean-style dry fermented sausages. *Foods* **2015**, *4*, 34–50. [CrossRef]
20. Tirloni, E.; Di Pietro, V.; Rizzi, G.; Pomilio, F.; Cattaneo, P.; Bernardi, C.; Stella, S. Non-thermal inactivation of *Listeria* spp. in a typical dry-fermented sausage "Bergamasco" salami. *Ital. J. Food Saf.* **2019**, *8*, 8112. [CrossRef]

21. Ranucci, D.; Miraglia, D.; Branciari, R.; Morganti, G.; Roila, R.; Zhou, K.; Jiang, H.; Braconi, P. Frankfurters made with pork meat, emmer wheat (*Triticum dicoccum* Schübler) and almonds nut (*Prunus dulcis* Mill.): Evaluation during storage of a novel food from an ancient recipe. *Meat Sci.* **2018**, *145*, 440–446. [CrossRef] [PubMed]
22. Dourou, D.; Porto-Fett, A.C.; Shoyer, B.; Call, J.E.; Nychas, G.J.E.; Illg, E.K.; Luchansky, J.B. Behavior of Escherichia coli O157: H7, *Listeria monocytogenes*, and *Salmonella typhimurium* in teewurst, a raw spreadable sausage. *Int. J. Food Microbiol.* **2009**, *130*, 245–250. [CrossRef] [PubMed]
23. Ranucci, D.; Branciari, R.; Acuti, G.; Della Casa, G.; Trabalza-Marinucci, M.; Miraglia, D. Quality traits of Ciauscolo salami from meat of pigs fed rosemary extract enriched diet. *Ital. J. Food Saf.* **2013**, *2*, e16. [CrossRef]
24. Branciari, R.; Balzano, M.; Pacetti, D.; Trabalza-Marinucci, M.; Della Casa, G.; Miraglia, D.; Capotorti, A.; Ranucci, D. Dietary CLA supplementation of pigs confers higher oxidative stability to Ciauscolo and Fabriano salami produced from their meat with no negative impact on physico-chemical, microbiological and sensorial characteristics. *Eur. J. Lipid. Sci. Technol.* **2016**, *118*, 1475–1485. [CrossRef]
25. Federici, S.; Ciarrocchi, F.; Campana, R.; Ciandrini, E.; Blasi, G.; Baffone, W. Identification and functional traits of lactic acid bacteria isolated from Ciauscolo salami produced in Central Italy. *Meat Sci.* **2014**, *98*, 575–584. [CrossRef]
26. Petruzzelli, A.; Blasi, G.; Masini, L.; Calza, L.; Duranti, A.; Santarelli, S.; Fisichella, S.; Pezzotti, G.; Aquilanti, L.; Osimani, A.; et al. Occurrence of *Listeria monocytogenes* in salami manufactured in the Marche Region (central Italy). *J. Vet. Med. Sci.* **2010**. [CrossRef]
27. Spanu, C.; Scarano, C.; Ibba, M.; Pala, C.; Spanu, V.; De Santis, E.P.L. Microbiological challenge testing for *Listeria monocytogenes* in ready-to-eat food: A practical approach. *Ital. J. Food Saf.* **2014**, *3*, 4518. [CrossRef]
28. European Commission. Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union L* **2015**, *338*, 1–26.
29. Branciari, R.; Valiani, A.; Franceschini, R.; Ranucci, D.; Lupattelli, A.; Urbani, E.; Ortenzi, R. Thermal inactivation and growth potential of *Listeria monocytogenes* in smoked tench. *Ital. J. Food Saf.* **2016**, *5*, 5974. [CrossRef]
30. Dalzini, E.; Cosciani-Cunico, E.; Pavoni, E.; Bertasi, B.; Daminelli, P.; Finazzi, G.; Losio, M.N.; Varisco, G. Study of growth potential of *Listeria monocytogenes* in low fat salami: An innovative Italian meat product. *Ital. J. Food Saf.* **2014**, *3*, 2112. [CrossRef]
31. European Union Reference Laborator. Technical guidance document for conducting shelf-life studies on Listeria monocytogenes in ready-to-eat foods. In Proceedings of the European Union Reference Laboratory for Listeria monocytogenes, Maisons–Alfort, France, 6 June 2014.
32. European Commission. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Off. J. Eur. Communities* **2008**, *50*, 18.
33. ISO 15214: 1998. Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Numeration of Mesophilic Lactic Acid Bacteria. Colony-Count Technique At 30 °C. Available online: https://www.iso.org/standard/26853.html (accessed on 26 June 2020).
34. Jaworska, D.; Neffe, K.; Kołożyn-Krajewska, D.; Dolatowski, Z. Survival during storage and sensory effect of potential probiotic lactic acid bacteria *Lactobacillus acidophilus* Bauer and *Lactobacillus casei* Bif3'/IV in dry fermented pork loins. *Int. J. Food Sci Tech.* **2011**, *46*, 2491–2497. [CrossRef]
35. AFNOR CERTIFICATION VALIDATION STUDY OF THE METHOD VIDAS *Listeria monocytogenes* II(LMO2 Ref.30122)BIO12/11 -03/04 for the detection of *Listeria monocytogenes*. Available online: https://nf-validation.afnor.org/wp-content/uploads/sites/2/2014/03/BIO-12-11-03-04_en.pdf (accessed on 26 June 2020).
36. UNI EN ISO 11290-2: 2005. Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of Listeria monocytogenes—Part 2: Enumeration method. Available online: https://www.iso.org/standard/25570.html (accessed on 26 June 2020).
37. Association of Official Analytical Chemists Inc. *Official Method of Analysis*, 17th ed.; Association of Official Analytical Chemists Inc.: Arlington, VA, USA, 2000.
38. Baranyi, J.; Roberts, T.A. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol. 1994 23*, 277–294. [CrossRef]

39. Dalzini, E.; Cosciani-Cunico, E.; Bernini, V.; Bertasi, B.; Losio, M.N.; Daminelli, P.; Varisco, G. Behaviour of *Escherichia coli* O157 (VTEC), *Salmonella typhimurium* and *Listeria monocytogenes* during the manufacture, ripening and shelf life of low fat salami. *Food Control.* **2015**, *47*, 306–311. [CrossRef]
40. Aquilanti, L.; Santarelli, S.; Silvestri, G.; Osimani, A.; Petruzzelli, A.; Clementi, F. The microbial ecology of a typical Italian salami during its natural fermentation. *Int. J. Food Microbiol.* **2007**, *120*, 136–145. [CrossRef]
41. Novelli, E.; Dal Santo, L.; Balzan, S.; Cardazzo, B.; Spolaor, D.; Lombardi, A.; Carraro, L.; Fasolato, L. Analysis of process factors of dry fermented salami to control *Listeria monocytogenes*. *Ital. J. Food Saf.* **2017**, *6*, 6184. [CrossRef]
42. Baranyi, J.; Tamplin, M.L. ComBase: A common database on microbial responses to food environments. *J. Food Prot.* **2004**, *67*, 1967–1971. [CrossRef]
43. Aguirre, J.S.; Rodríguez, M.R.; de Fernando, G.D.G. Effects of electron beam irradiation on the variability in survivor number and duration of lag phase of four food-borne organisms. *Int. J. Food Microbiol.* **2011**, *149*, 236–246. [CrossRef]
44. Fridman, O.; Goldberg, A.; Ronin, I.; Shoresh, N.; Balaban, N.Q. Optimization of lag time underlies antibiotic tolerance in evolved bacterial populations. *Nature* **2014**, *513*, 418–421. [CrossRef]
45. Li, B.; Qiu, Y.; Shi, H.; Yin, H. The importance of lag time extension in determining bacterial resistance to antibiotics. *Analyst* **2016**, *141*, 3059–3067. [CrossRef]
46. Ross, T.; Dalgaard, P.; Tienungoon, S. Predictive modelling of the growth and survival of Listeria in fishery products. *Int. J. Food Microbiol.* **2000**, *62*, 231–245. [CrossRef]
47. Cole, M.B.; Jones, M.V.; Holyoak, C. The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *J. Appl. Bacteriol.* **1990**, *69*, 63–72. [CrossRef] [PubMed]
48. Sabatakou, O.; Watso, E.; Mantis, F.; Ramantanis, S. Classification of Greek meat products on the basis of pH and aw values. *Fleischwirtschaft (Frankfurt)* **2001**, *81*, 91–95.
49. Nyachuba, D.G.; Donnelly, C.W.; Howard, A.B. Impact of nitrite on detection of *Listeria monocytogenes* in selected ready-to-eat (RTE) meat and seafood products. *J. Food Sci.* **2007**, *72*, M267–M275. [CrossRef] [PubMed]
50. Devlieghere, F.; Geeraerd, A.H.; Versyck, K.J.; Vandewaetere, B.; Van Impe, J.; Debevere, J. Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: A predictive model. *Food Microbiol.* **2001**, *18*, 53–66. [CrossRef]
51. Faleiro, M.L.; Andrew, P.W.; Power, D. Stress response of *Listeria monocytogenes* isolated from cheese and other foods. *Int. J. Food Microbiol.* **2003**, *84*, 207–216. [CrossRef]
52. Reis, J.A.; Paula, A.T.; Casarotti, S.N.; Penna, A.L.B. Lactic acid bacteria antimicrobial compounds: Characteristics and applications. *Food Eng. Rev.* **2012**, *4*, 124–140. [CrossRef]
53. Ranucci, D.; Roila, R.; Andoni, E.; Braconi, P.; Branciari, R. Punica granatum and Citrus spp. Extract Mix Affects Spoilage Microorganisms Growth Rate in Vacuum-Packaged Cooked Sausages Made from Pork Meat, Emmer Wheat (*Triticum dicoccum* Schübler), Almond (*Prunus dulcis* Mill.) and Hazelnut (*Corylus avellana* L.). *Foods* **2019**, *8*, 664. [CrossRef]
54. Rowan, N.J.; Anderson, J.G.; Smith, J.E. Potential infective and toxic microbiological hazards associated with the consumption of fermented foods. In *Microbiology of Fermented Foods*; Springer: Boston, MA, USA, 1998; pp. 812–837.
55. Leroy, F.; De Vuyst, L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci. Technol.* **2004**, *15*, 67–78. [CrossRef]
56. Foulquié Moreno, M.R.; Callewaert, R.; Devreese, B.; Van Beeumen, J.; De Vuyst, L. Isolation and biochemical characterisation of enterocins produced by enterococci from different sources. *J. Appl. Microbiol.* **2003**, *94*, 214–229. [CrossRef]
57. Al-Zeyara, S.A.; Jarvis, B.; Mackey, B.M. The inhibitory effect of natural microflora of food on growth of *Listeria monocytogenes* in enrichment broths. *Int. J. Food Microbiol.* **2011**, *145*, 98–105. [CrossRef] [PubMed]
58. Cornu, M.; Billoir, E.; Bergis, H.; Beaufort, A.; Zuliani, V. Modeling microbial competition in food: Application to the behavior of *Listeria monocytogenes* and lactic acid flora in pork meat products. *Food Microbiol.* **2011**, *28*, 639–647. [CrossRef] [PubMed]
59. Belfiore, C.; Fadda, S.; Raya, R.; Vignolo, G. Molecular basis of the adaption of the anchovy isolate *Lactobacillus sakei* CRL1756 to salted environments through a proteomic approach. *Food Res. Int. 2013 54*, 1334–1341. [CrossRef]

60. Aquilanti, L.; Garofalo, C.; Osimani, A.; Clementi, F. Ecology of lactic acid bacteria and coagulase negative cocci in fermented dry sausages manufactured in Italy and other Mediterranean countries: An overview. *Int. Food Res. J.* **2016**, *23*, 429–445.
61. Martinez, R.C.R.; Staliano, C.D.; Vieira, A.D.S.; Villarreal, M.L.M.; Todorov, S.D.; Saad, S.M.I.; de Melo Franco, B.D.G. Bacteriocin production and inhibition of *Listeria monocytogenes* by *Lactobacillus sakei* subsp. sakei 2a in a potentially synbiotic cheese spread. *Food Microbiol.* **2015**, *48*, 143–152. [CrossRef] [PubMed]
62. Amadoro, C.; Rossi, F.; Piccirilli, M.; Colavita, G. Features of *Lactobacillus sakei* isolated from Italian sausages: Focus on strains from Ventricina del Vastese. *Ital. J. Food Saf.* **2015**, *4*, 5449. [CrossRef]
63. Fontana, C.; Cocconcelli, P.S.; Vignolo, G.; Saavedra, L. Occurrence of antilisterial structural bacteriocins genes in meat borne lactic acid bacteria. *Food Control* **2015**, *47*, 53–59. [CrossRef]
64. Casaburi, A.; Di Martino, V.; Ferranti, P.; Picariello, L.; Villani, F. Technological properties and bacteriocins production by *Lactobacillus curvatus* 54M16 and its use as starter culture for fermented sausage manufacture. *Food Control.* **2016**, *59*, 31–45. [CrossRef]
65. Jones, R.J.; Zagorec, M.; Brightwell, G.; Tagg, J.R. Inhibition by *Lactobacillus sakei* of other species in the flora of vacuum packaged raw meats during prolonged storage. *Food Microbiol.* **2009**, *26*, 876–881. [CrossRef]
66. Nyquist, O.L.; McLeod, A.; Brede, D.A.; Snipen, L.; Aakra, Å.; Nes, I.F. Comparative genomics of *Lactobacillus sakei* with emphasis on strains from meat. *Mol. Genet. Genom.* **2011**, *285*, 297–311. [CrossRef]
67. Müller, D.M.; Carrasco, M.S.; Tonarelli, G.G.; Simonetta, A.C. Characterization and purification of a new bacteriocin with a broad inhibitory spectrum produced by *Lactobacillus plantarum* lp 31 strain isolated from dry-fermented sausage. *J. Appl. Microbiol.* **2009**, *106*, 2031–2040. [CrossRef] [PubMed]
68. Nielsen, D.S.; Cho, G.S.; Hanak, A.; Huch, M.; Franz, C.M.; Arneborg, N. The effect of bacteriocin-producing *Lactobacillus plantarum* strains on the intracellular pH of sessile and planktonic *Listeria monocytogenes* single cells. *Int. J. Food Microbiol.* **2010**, *141*, S53–S59. [CrossRef] [PubMed]
69. Barbosa, M.S.; Todorov, S.D.; Ivanova, I.V.; Belguesmia, Y.; Choiset, Y.; Rabesona, H.; Chobert, J.M.; Haertlè, T.; Franco, B.D.G.D.M. Characterization of a two-peptide plantaricin produced by *Lactobacillus plantarum* MBSa4 isolated from Brazilian salami. *Food Control.* **2016**, *60*, 103–112. [CrossRef]
70. Maldonado, A.; Ruiz-Barba, J.L.; Jiménez-Díaz, R. Purification and genetic characterization of plantaricin NC8, a novel coculture-inducible two-peptide bacteriocin from *Lactobacillus plantarum* NC8. *Appl. Environ. Microbiol.* **2003**, *69*, 383–389. [CrossRef]
71. Kingcha, Y.; Tosukhowong, A.; Zendo, T.; Roytrakul, S.; Luxananil, P.; Chareonpornsook, K.; Valyansevi, R.; Sonomoto, K.; Visessanguan, W. Anti-listeria activity of *Pediococcus pentosaceus* BCC 3772 and application as starter culture for Nham, a traditional fermented pork sausage. *Food Control.* **2012**, *25*, 190–196. [CrossRef]
72. Leistner, L. Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol.* **2000**, *55*, 181–186. [CrossRef]
73. Branciari, R.; Avellini, P.; Sukasi-Sangamayya, R.; Di Antonio, E.; Rea, S. PCR-RFLP analysis (Polymerase chain reaction-restriction fragment. *Ind. Aliment.* **2000**, *39*, 313–318.