In vitro Modelling of Simultaneous Interactions of *Listeria monocytogenes*, *Lactobacillus sakei*, and *Staphylococcus carnosus*

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Abstract The Jameson effect model describes suppression of microorganism growth as being dependent on non-specific competition. This model was developed for simultaneous growth in a liquid medium and microbial interactions between *Listeria monocytogenes*, *Lactobacillus sakei*, and *Staphylococcus carnosus* with addition of NaNO₂ to mimic the manufacturing process of salami for 48 h at 21°C and then for 14 days at 17°C. *L. monocytogenes* in the presence of *L. sakei* was inhibited by 2.120 log CFU/mL in the presence of NaNO₂, and 1.146 log CFU/mL without NaNO₂. Inhibition of *L. monocytogenes* co-cultured with *S. carnosus* was 2.248 log CFU/mL at 48 h, but after 336 h was not significantly ($p>0.05$) different from *L. monocytogenes* in mono-culture. The interspecific competition parameter (β) <1 indicated that the prevailing competition in co-cultures was intraspecific. Differentiation between 2 bacterial species interactions can be applied for use in starter cultures with pathogenic flora.

Keywords: Jameson effect, co-culture, *Listeria monocytogenes*, *Lactobacillus sakei*, *Staphylococcus carnosus*

Introduction

The usefulness of predictive microbiology models for determination of processing control and minimization of risks associated with food safety has been known since the 1990s. Baranyi and Roberts (1) noted that models contributed to an understanding of physiological relationships and also for consideration of individual bacterial and population behaviors (2,3). It is useful to understand interactions in bacterial populations through Jameson-based effect models, which can be described as competition between bacterial species for limited resources in a given environment for maximization of growth and population size (4). When resources are depleted, growth of all co-cultured bacterial species ceases. According to Cornu *et al.* (5), many microbial interactions are limited to a cell concentration reduction and a minor population decrease when the major population reaches a maximum level. Studies of *L. monocytogenes* by World Health Organization – Food and Agriculture Organization of the United Nations (WHO-FAO) (6) and Gnanou-Besse *et al.* (7) in which the cell concentration of *L. monocytogenes* (minor population) was partly inhibited by limited resources and inhibition of waste products when lactic acid bacteria (LAB) reached a stationary phase

(major population) showed that growth stopped.

Although the Jameson effect has been described in 2 bacterial populations, modelling between 3 bacterial species might also be useful when 2 species present an antagonist potential against pathogenic bacteria. Martínez and De Martinis (8), Winkelströter *et al.* (9), and Wang *et al.* (10) reported beneficial effects of starter culture applications in food, such as an ability to inhibit growth, adhesion, and biofilm formation of *L. monocytogenes*. Nonetheless, these studies were not based on mathematical model approximations. Reported antagonistic mechanisms of starter cultures on *L. monocytogenes* may be explained by competition for nutrients and essential factors, and by production of acid, bacteriocins, enzymes, and secondary metabolites. *L. monocytogenes* is capable of surviving in foods for long periods of time (11). For this reason, control of this human pathogen is important because of the severity of listeriosis (12).

According to Ray and Daeschel (13), more than 100 different isolated species of the genus *Lactobacillus* exist as native microbiota in food. *L. sakei* is a dominant species in fermented meat products and is also widely used as a starter culture. Tran *et al.* (14) identified this species in 89% of ripened cold meats analyzed in Europe. Apart

from native microbiota in meat products, coagulase-negative cocci (CNC) are also relevant and are involved in color development and stability through reduction of nitrite levels, which eventually leads to formation of nitrosomyoglobin and contributes to control of lipid oxidation (15,16). *Staphylococcus carnosus* is a facultative anaerobe CNC that can survive under stressful conditions of high salt concentrations and low temperatures. *S. carnosus* has been observed during meat fermentation (17). Co-cultures of *S. carnosus* and *L. sakei* can secure the sensory quality of raw-cured products based on metabolic functions of both species. Leroy *et al.* (18) reported that *L. sakei* co-cultured with *S. carnosus* generated an effective fermentation process in sausage processing in which *S. carnosus* is competitive in the initial stage of fermentation, then decreases in effectiveness during ripening. The objective of this study was to model simultaneous growth of co-cultures in liquid medium and microbial interactions between *L. monocytogenes*, *L. sakei*, and *S. carnosus* based on the Jameson effect under 2 temperatures to mimic the manufacturing process of salami with NaNO₂ addition.

Materials and Methods

Bacterial strains *L. sakei* and *S. carnosus* were isolated from the commercial starter culture (T-SC-150 Bactoform; CHR-Hansen, Hoersholm, Denmark). Identities of strains were verified using PCR reactions for amplification of a hypervariable region of the 16S rRNA gene. PCR products were sequenced at Macrogen, Seoul, Korea. Sequences were edited using Chromas Lite 2.1.1 (Technelysium, Brisbane, Australia) and analyzed using to genomic database (Basic Local Alignment Search Tool, BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequencing results were compared with sequences contained in the GenBank non-redundant database, which returned hits including a strain of *Lactobacillus sakei* with 99.9% similarity and *Staphylococcus carnosus* strain with 100% similarity. *L. monocytogenes* strain ATCC 19115 was maintained in glass beads with glycerol/Nutrient Broth (Scharlau Chemie SA, Barcelona, Spain) (20/80%) (v/v) and stored at -80°C in liquid nitrogen.

Inoculum preparation, media, and incubation conditions Each strain was cultured twice successively with glass beads in 100 mL of BHI medium (Scharlau Chemie SA) modified by addition of 0.25% glucose (Merck, Darmstadt, Germany) and incubated (Friocell 22-Comfort; MMM group, Munich, Germany) for 48 h at 37±1°C without agitation for inoculation in co-culture testing. For all plate count experiments, 6 decimal dilutions were performed for each strain sample (1 mL) in triplicate using 0.1% peptone water (10 mL) (Merck) in sterile Eppendorf tubes with vortex agitation for 30 s (standard 541; Heidolph Instruments Labortechnik, Schwabach, Germany) between dilutions, followed by plating on selective media. For *L. sakei*, Man, Rogosa and Sharpe agar (MRS) (Scharlau Chemie SA) was subjected to addition of 4 mg amoxicillin and 2 mg clavulanic

acid per L of agar (MRS) (Scharlau Chemie SA). Baird Parker agar (Scharlau Chemie SA) with egg yolk tellurite potassium (Scharlau Chemie SA) was used for *S. carnosus*, and polymyxin-acriflavine-LiCl-ceftazidime-aesculin-mannitol agar (PALCAM) (Scharlau Chemie SA) with Listeria selective supplement (Oxoid, UK) was used for *L. monocytogenes*. Inocula were incubated (Friocell 22-Comfort; MMM group) at 37±1°C for 48 h and posterior counts were recorded.

Growth experiments in co-culture Simultaneous growth in co-culture was evaluated in a complete factorial design with 12 treatments in total per bacterial species with 9 replicates, and 0 and 50 ppm levels of NaNO₂ addition for all conditions, 3 **mono-culture conditions**: *L. monocytogenes* in monoculture (LM, LM⁺), *L. sakei* in monoculture (LS, LS⁺), *S. carnosus* in monoculture (SC, SC⁺) and 3 **co-culture conditions**: *L. monocytogenes* in co-culture with *L. sakei* and *S. carnosus* (L-B, L-B⁺), *L. sakei* in co-culture with *L. monocytogenes* and *S. carnosus* (LS-B, LS-B⁺) and *S. carnosus* in co-culture with *L. monocytogenes* and *L. sakei* (SC-B, SC-B⁺), Superscript (+) corresponds to treatments with 50 ppm sodium NaNO₂.

Each strain was activated under identical conditions described above for evaluation of microbial population dynamics. For all treatments, cultures were placed in 15 mL Falcon tubes and the volume was brought to a 5 mL final volume using sterile BHI broth modified using addition of 0.25% w/w glucose. Cultures were inoculated when 650 µL of *L. sakei* reached an OD_{600 nm} value of Abs 1.5 (Bioscreen C; Oy Growth Curves Ab Ltd., Helsingfors, Finland) and 580 µL of *S. carnosus* reached an OD_{600 nm} value of Abs 1.2, which both corresponded to approximately 6 log CFU/mL, and 50 µL of the second dilution of *L. monocytogenes* reached an OD_{600 nm} value of Abs 1.3, which corresponded to approximately 3 log CFU/mL. Experiments were performed at temperatures of 21±0.1°C for 0-48 h and at 17±0.1°C from 48 h to 14 days aerobically without agitation. Temperatures at which experiments were undertaken were normal for the fermentation and ripening processes of salami and promoted challenge organism testing in co-culture. Each microorganism was counted on agar plates under conditions described above at 0, 3, 6, 9, 12, 16, 20, 24, 48, 144, 192, and 336 h.

Modelling

Primary growth model: The Baranyi and Roberts (19) model was used for determination of the maximum growth rate ($\mu_{\max}$) due to consideration and reconciliation of bacterial behavior both individually and as a population, based on the intracellular conditions that define the physiological state of cells according to history and extracellular conditions. The Baranyi and Roberts model is used for generation of $\mu_{\max}$ based on the assumption that the growth rate is a parameter that describes cell concentration variations over time and, thus, produces a line that tends to be straight in the exponential growth phase (1,2). Data from all experimentally obtained individual growth curves for each strain were fitted to the model of Baranyi and Roberts (19). For this procedure, the Macro DMFit version 3.5 Excel[®]

add-in (www.ifr.ac.uk/safety/DMfit) (Baranyi and Roberts, Institute of Food Research, Norwich) was used.

The equation proposed by Dalgaard (20) was used for determination of the lag phase (λ) due to consideration of the adaptation period. Therefore, cells are physiologically modified and express metabolic capabilities in the time required for the metabolic machinery of the cell to generate sufficient energy to begin replication. The lag phase (λ) was estimated based on equation 1 (Eq. 1) (20), where t_i corresponds to the time when the cell concentration is equal to $N_{\max}/2$ ($N_{\max}$ =maximum population density), $N_{\min}$ corresponds to the minimum population density, and e corresponds to an exponential function. MATLAB R2010a software (The MathWorks USA) was used.

$$\lambda = t_i - \frac{1}{\mu_{\max}} \ln \left(\frac{N_{\max} + N_{\max}(e^{\mu_{\max} t_i})}{N_{\max} + N_{\min}(e^{\mu_{\max} t_i})} - 1 \right) \quad (1)$$

Primary growth models were fitted to monoculture treatments to obtain growth parameters for all individual curves of tested microorganisms obtained in co-culture testing.

Inter-bacterial interaction model: Suppression of microorganism growth dependent on non-specific competition between 3 microorganisms as described by the Jameson model was tested where the first organism to reach a maximum population density suppressed growth of competitors due to substrate depletion. Differences in growth rates allow a dominant strain to reach the stationary phase first. The proposed model (Eq. 2) was based on the model described by Powell (21) and Giménez and Dalgaard (22), and was developed based on the Jameson effect using parameters of cell concentration (N), physiological state of bacteria populations (Q), and interspecific competition terms (β_{xy}). These parameters corresponded to the influence of bacteria species_x on species_y, where subscripts (_{LS}), (_{SC}), and (_{LM}) corresponded to *L. sakei*, *S. carnosus*, and *L. monocytogenes*, respectively. The superscript (^{max}) corresponded to the variable evaluated in a monoculture of each species. A numerical solution was calculated using the fourth-order Runge-Kutta method for numerical integration of differential equations. MATLAB R2010a (The MathWorks) was used.

$$\frac{dN_{LS}}{dt} = \mu_{LS}^{\max} N_{LS} \frac{Q_{LS}}{1 + Q_{LS}} \left(1 - \frac{N_{LS} + \left(\beta_{LS} \cdot N_{SC} \right) + \left(\beta_{LS} \cdot N_{LM} \right)}{N_{LS}^{\max}} \right) \quad (2A)$$

$$\frac{dN_{SC}}{dt} = \mu_{SC}^{\max} N_{SC} \frac{Q_{SC}}{1 + Q_{SC}} \left(1 - \frac{N_{SC} + \left(\beta_{SC} \cdot N_{LS} \right) + \left(\beta_{SC} \cdot N_{LM} \right)}{N_{SC}^{\max}} \right) \quad (2B)$$

$$\frac{dN_{LM}}{dt} = \mu_{LM}^{\max} N_{LM} \frac{Q_{LM}}{1 + Q_{LM}} \left(1 - \frac{N_{LM} + \left(\beta_{LM} \cdot N_{SC} \right) + \left(\beta_{LM} \cdot N_{LS} \right)}{N_{LM}^{\max}} \right) \quad (2C)$$

Statistical analysis Growth parameters $N_{\max}$, $\mu_{\max}$, and λ were

compared using models determined for each treatment with an *F* test, assuming quantitative approach planned testing with orthogonal contrasts at $p < 0.05$. SAS 9.2 (SAS Institute Inc., Cary, NC, USA) was used. Model fit was based on the Root Mean Square error (RMSE) following the method of Ratkowsky (23). RMSE is a measure of the goodness-of-fit for both linear and non-linear models.

This study did not involve any experimentation with human or animal subjects performed by any of the authors.

Results and Discussion

Inter-bacterial behavior in co-culture testing *L. monocytogenes* with *L. sakei* presented an inhibition of 1.146 ± 0.188 log CFU/mL without NaNO_2 (Fig. 1A) and an inhibition of 2.120 ± 0.579 log CFU/mL in the presence of 50 ppm NaNO_2 (Fig. 1B). Both values were significantly ($p < 0.05$) different from control condition values. On the other hand, inhibition was dose-dependent because this behavior was not evident in a co-culture of *L. monocytogenes* with *L. sakei* and *S. carnosus* at average initial concentrations of 4.762 ± 0.196 log CFU/mL for *L. sakei* and 4.625 ± 0.138 log CFU/mL for *S. carnosus* (data not shown).

For treatments lacking NaNO_2 , inhibition of 2.248 ± 0.548 log CFU/mL at 48 h for *L. monocytogenes* co-cultured with *S. carnosus* was observed (Fig. 1A). Tjener *et al.* (24) and Leroy *et al.* (18) determined that *S. carnosus* was more competitive in the initial 48 h than later. The population density of *L. monocytogenes* after 336 h of co-culture with *S. carnosus* was not significantly different ($p > 0.05$) from before 336 h because viable counts of *S. carnosus* were constant during this period.

For treatments with 50 ppm NaNO_2 , there was significantly ($p < 0.05$) greater inhibition of *L. monocytogenes* co-cultured with *L. sakei* (Fig. 1B), compared with treatments lacking NaNO_2 (Fig. 1A). Thus, there was no difference in total counts of *L. monocytogenes* between 48 and 336 h, mimicking the ripening processing, which prevented identification of maximum levels of antimicrobial activity.

There was greater inhibition of *L. monocytogenes* with *L. sakei* in treatments with NaNO_2 . Sebranek and Bacus (25) showed that cellular respiration could be inhibited by interaction of nitrite with the heme-enzyme of the cytochrome system which, under anaerobic conditions, could inactivate sulfhydryl groups of enzymes in the pyruvate metabolism, thus limiting *L. monocytogenes* growth. Nitrite inhibits anaerobic respiration through active transport of glucose and proline, and oxidative phosphorylation and nitric oxide inactivates ferredoxin reductase, which is involved in generation of ATP (25). Thus, inhibition of *L. monocytogenes* by approximately 1 log cycle was achieved in this study, in agreement with results of Martínez and De Martinis (8) where *in vitro* inhibition in BHI broth was reported. It is possible that the most important factor for inhibition of *L. monocytogenes* under the *in vitro* experimental conditions herein was inhibition by competition for nutrients and essential growth factors because the 5% NaCl concentration used in

Fig. 1. *L. monocytogenes* behavior in co-culture without sodium nitrite (A); 50 ppm sodium nitrite (B). Control monoculture treatments in monoculture (●). Co-culture with *L. sakei* (▨), *S. carnosus* (▲), *L. sakei* and *S. carnosus* (○). Error bars indicate standard deviation (SD).

Fig. 2. *L. sakei* behavior in co-culture without sodium nitrite (A); 50 ppm sodium nitrite (B). Control monoculture treatments in monoculture (●). Co-culture with *L. monocytogenes* (▨), *L. monocytogenes* and *L. sakei* (○). Error bars indicate SD.

BHI broth may have interfered with production of bacteriocins. Leroy and De Vuyst (26) reported similar conclusions regarding a strong influence of the NaCl concentration on production of bacteriocins where concentrations of 6% NaCl produced interference with induction factor links associated with production of bacteriocins.

Reduction in cell concentration of *L. monocytogenes* might have been due to generation of organic compounds, such as lactic acid, hydrogen peroxide (H₂O₂), catalase, and other proteolytic enzymes. Leroy and De Vuyst (26) indicated that *L. sakei* had a higher production rate of lactic acid of 30 g/L after 30 h in MRS broth at 30°C, compared with other microorganisms with a bioprotection potential, such as *L. curvatus*, *L. amylovorus*, and *Enterococcus faecium*. According to Vermeiren *et al.* (27), inhibition cannot be explained solely based on a decrease in pH resulting from generation of lactic acid because the optimum pH for *L. monocytogenes* growth

is between 4.7 and 9.2. In this study, the pH at 336 h for treatments without NaNO₂ was 4.687±0.031, and the pH was 4.857±0.047 with addition of 50 ppm NaNO₂ (data not shown).

L. sakei can also produce H₂O₂ in the presence of O₂, which has an effect against spoilage bacteria in food (28). In addition, *L. sakei* can also protect the quality of products against defects in color and a stale taste (29). *L. sakei* has a heme-dependent complex of active catalase in meat systems that reduces H₂O₂ levels, and *L. sakei* also lacks catabolic pathways of tyrosine histamine (30). Additionally, during competition, *L. sakei* can deprive pathogens of sugars or essential developmental components, such as iron. Elli *et al.* (31) indicated that *L. monocytogenes* is developmentally impaired in the absence of iron because iron acts as a signal that activates expression of proteins that are necessary for survival and colonization. Moreover, Winkelströter *et al.* (9) indicated that *L. sakei* reduces the

Fig. 3. *S. carnosus* behavior in co-culture without sodium nitrite (A); 50 ppm sodium nitrite (B). Control monoculture treatments in monoculture (●). Co-culture with *L. monocytogenes* (▲), *L. monocytogenes* and *S. carnosus* (○). Error bars indicate SD.

Table 1. Growth parameters estimation and the model-fitted measure in co-culture treatments

Treatment	$\mu_{\max}$ (1/h)	λ (h)	Model fitted measure
			RMSE
LM	1.060±0.11	27.66±0.1	
LM ⁺	0.750±0.17	32.94±0.2	
L-B	0.462±0.02	-	0.746
L-B ⁺	0.437±0.02	-	0.327
P-Contrast*	a	0.30	
	b	0.03	
LS	0.028±0.003	-	
LS ⁺	0.053±0.002	12.98±0.3	
LS-B	0.012±0.002	-	1.004
LS-B ⁺	0.009±0.001	32.18±1.5	1.357
P-Contrast*	a	0.02	
	b	0.004	0.87
SC	0.002±0.0007	-	
SC ⁺	0.002±0.0001	-	
SC-B	0.001±0.0001	-	1.202
SC-B ⁺	0.001±0.0001	-	1.364
P-Contrast*	a	0.25	
	b	0.007	

Treatments⁺ were evaluated with addition of 50 ppm NaNO₂.

- parameter was not calculated ($\lambda < 3$ h)

Treatments: *L. monocytogenes* in monoculture (LM), *L. sakei* in monoculture (LS), *S. carnosus* in monoculture (SC), *L. monocytogenes* in co-culture with *L. sakei* and *S. carnosus* (L-B), *L. sakei* in co-culture with *L. monocytogenes* and *S. carnosus* (LS-B) and *S. carnosus* in co-culture with *L. monocytogenes* and *L. sakei* (SC-B).

**p* value for orthogonal contrast. *a*=Treatments in co-culture with addition of 50 ppm NaNO₂ vs. treatments in co-culture without NaNO₂; *b*=Treatments in monoculture vs. treatments in co-culture.

ability of *L. monocytogenes* to adhere and form biofilms. *S. carnosus* was not inhibited by growth of *L. monocytogenes* and numbers remained constant from 48 to 336 h (14 days). This behavior was

Table 2. Interspecific competition parameters in co-culture treatments

	NaNO ₂ Addition (ppm)	
	50	0
$\beta_{LS/SC}$ ¹⁾	0.118	0.159
$\beta_{LS/L}$	0.12	0.098
$\beta_{SC/LS}$	0.12	0.11
$\beta_{SC/L}$	0.1	0.084
$\beta_{L/LS}$	0.264	0.137
$\beta_{L/SC}$	0.183	0.16

¹⁾*L. monocytogenes* (L), *L. sakei* (LS), *S. carnosus* (SC).

similar to other reports for fermented meat products (18,24,32) and probably indicates that *S. carnosus* used as a starter culture can be useful for improving the sensory characteristics of products, such as salami.

Inter-bacterial interaction model The underlying mechanism of the Jameson effect appeared to be growth parameter changes with different responses in co-culture combinations under 0 and 50 ppm NaNO₂. For L-LS treatments, *L. sakei* reached a maximum bacterial population and initiated the stationary phase at 48 h (Fig. 2A), whereas the *L. monocytogenes* population decreased (Fig. 1A). However, at 96 h, *L. monocytogenes* reached a microbial density similar to *L. sakei* just as the *L. sakei* population began to decrease. Moreover, in L-LS⁺ treatments, when *L. monocytogenes* and *L. sakei* reached the same population density at 96 h, both populations began to decrease, probably because of an inhibitory effect of NaNO₂ on *L. monocytogenes* (Fig. 1B and 2B). However, this situation was insufficient for maintenance of *L. monocytogenes* inhibition in this co-culture system.

L. monocytogenes and *L. sakei* reached the same population density at 96 h and both populations begin to decrease, showing simultaneous deceleration of 2 microbial populations. This behavior

Fig. 4. Behavior curves in co-culture without sodium nitrite (A); and 50 ppm sodium nitrite (B). Real data sets for *L. monocytogenes* ($\square$), *L. sakei* ($\triangle$) and *S. carnosus* ($\circ$). Estimation by model (Eq. 2) for *L. monocytogenes* (—), *L. sakei* (---) and *S. carnosus* (-.-).

corresponded to an approach based on the Jameson effect hypothesis and provided a phenomenological description of microorganism growth in the presence of other bacterial species due to the bacterial interaction. The Lotka-Volterra model according to Giuffrida *et al.* (33) and Cornu *et al.* (5) simulates competition between 2 species and describes different interactions for reduction of only a single population, related to the bacterial concentration, depends only on environmental conditions since interaction parameters do not affect the maximum growth rate.

Results for *L. monocytogenes* co-cultured with *L. sakei* in this study were in agreement with a report of Mellefont *et al.* (4) where co-culture of *L. monocytogenes* (7.87 ± 0.04 log CFU/mL) with *L. plantarum* (9.18 ± 0.06 log CFU/mL), a bacterium typically used as a starter culture without a specific inhibition effect on *L. monocytogenes*, slowed the increase in *L. monocytogenes* cell population after a short period of suppression concomitant with a decline in *L. plantarum* numbers. It is possible that the *L. plantarum* decline was accompanied by cell lysis, thus releasing nutrients that could be used by *L. monocytogenes*, enabling a higher *L. monocytogenes* population density.

For L-SC and L-SC⁺ treatments, *S. carnosus* reached a maximum density population at 24 h. However, *L. monocytogenes* growth continued so that the 2 co-cultured populations reached the same population density after 192 h and remained in the stationary phase thereafter (Fig. 3A and 3B). Thus, nutrient availability did not contribute to an inhibition effect under these conditions.

In an *L. monocytogenes* co-culture with *S. carnosus*, growth of both strains continued and reached the same population density, in this case there was no depletion effect. According to Mellefont *et al.* (4), the depletion effect only leads to suppression if a nutrient is essential for growth and no alternative substrates are available for bacterial populations. Furthermore, *S. carnosus* is not usually

considered to produce specific growth inhibitors against pathogens. Therefore, *S. carnosus* addition to some meat products is usually aimed at improving the flavor and color.

L. monocytogenes co-cultured with *L. sakei* and *S. carnosus* in L-B treatments resulted in all species achieving the same density after 6 h. Subsequently, *L. monocytogenes* continued to grow, numbers of *L. sakei* began to decrease, and *S. carnosus* continued in the stationary phase. However, for L-B⁺ treatments, all populations continued to increase once the 3 species reached the same density. When *L. monocytogenes* achieved a maximum density population, numbers of *L. sakei* began to decrease and *S. carnosus* continued in the stationary phase. These behaviors were similar for the L-LS, L-LS⁺, L-SC, and L-SC⁺ treatments. Based on the importance of the growth rate ($\mu_{\max}$) in co-culture experiments (Table 1), although *L. monocytogenes* was not numerically dominant in the initial inoculum, it had the highest $\mu_{\max}$ value of the bacterial species tested, which probably lead to dominance in the final population. *L. monocytogenes* co-cultured with *L. sakei* and *S. carnosus* (Table 1) showed a significantly ($p < 0.05$) lower $\mu_{\max}$ value than in a monoculture control. Thus, inhibition did not result from addition of 50 ppm NaNO₂ because inhibition did not differ significantly ($p > 0.05$) with and without nitrite treatments.

The greatest influences on growth were addition of NaNO₂ and a decrease of the $\mu_{\max}$ value for *L. sakei*, whereas a positive effect was observed in co-cultures with *S. carnosus*. For *S. carnosus*, the $\mu_{\max}$ value was not positively influenced by addition of 50 ppm NaNO₂, but growth parameters showed significant differences ($p < 0.01$) under mono and co-culture conditions with an increase in the $\mu_{\max}$ value during co-culture with *L. sakei*. In this study, the $N_{\max}$ value for each microorganism in monoculture was assumed, and the analysis was simplified using the average $N_{\max}$ value (4). Results of model fitting are shown in Table 1. The model (Fig. 4) was based on

coexistence of 3 species in the culture medium as $N_{L-B}/\beta_{L/S} > N_{L-S-B}$; $N_{L-B}/\beta_{L/S} > N_{S-B}$. The interspecific competition parameter (β) < 1 indicated that the prevailing competition in the co-culture was intraspecific (Table 2). Therefore, the model was based on the hypothesis that *L. sakei* and *S. carnosus* dominated the competition, where respective concentrations tended to approach the population densities seen in monoculture, and exerted an influence on the dynamics of *L. monocytogenes* growth. Therefore, the Jameson-based model (Eq. 2) arose due to competitive exclusion; however the results indicate that the species could coexist.

Cell concentration of *L. sakei* were significantly reduced ($p < 0.05$) in co-culture with *L. monocytogenes* (Fig. 2A and 2B), probably due to the competitive effect for nutrients under the principle of competitive exclusion in an interspecific relationship of amensalism (34). Although the population density of *L. sakei* in co-culture with *S. carnosus* and *L. monocytogenes* was maintained, there were significant differences in the population density of *L. sakei* ($p < 0.05$). Model-fitted measures showed that the Jameson-based model (Eq. 2) for *L. monocytogenes* behavior was comparable both with and without NaNO_2 addition (RMSE=0.327 and 0.746, respectively) (Table 1). Thus, a fundamental role was attributable to interspecific competition for the bacterial dynamics of *L. monocytogenes* when co-cultured with *L. sakei* and *S. carnosus*. In addition, the RMSE measure indicated that the model did tend toward underestimation for cell concentration of *L. monocytogenes* and that the model based on the Jameson effect led to an overestimation of *L. sakei* cell concentration (RMSE=1.357 for treatments with 50 ppm NaNO_2 and RMS=1.004 for treatments without NaNO_2), and *S. carnosus* cell concentration (RMSE=1.364 for treatments with 50 ppm NaNO_2 and RMSE=1.202 for treatments without NaNO_2). Thus, the Jameson-based model could not be used to explain the complexity of the microbiological system (Fig. 4). Therefore, an increase in the RMSE value, which describes competition based mainly on nutrient kinetics (5) and incorporates the microbial inactivation phase, was proposed.

In treatments of *L. sakei* co-cultured with *S. carnosus* and *L. monocytogenes*, the population density of *L. sakei* was stable. According to Verluyten *et al.* (35), oxidative stress generated under aerobic conditions can induce a protective response in *L. sakei*, thus requiring additional expense of ATP that, therefore, cannot be used for bacterial growth. An interspecific, mutualistic relationship between *L. sakei* and *S. carnosus* was shown by the population increase of *L. sakei* and the increase in the μ_{max} value, but no increase in *S. carnosus* cell numbers. Smid and Lacroix (34) indicated that cells in co-culture offer greater resistance to inhibition generated by other bacteria and greater tolerance to variations in environmental factors, compared with bacterial monocultures. This occurs because microbial consortia can communicate via metabolites and other signalling molecules. Therefore, each cell in a co-culture responds to the presence of other cells.

In conclusion, cell behavior in a co-culture under 2 temperature conditions can be similar to what occurs during fermentation in meat

products. *L. sakei* can be used in a protective culture for growth control of *L. monocytogenes*. Moreover, when a protective culture was used, a decrease in *L. monocytogenes* counts was observed in treatments with NaNO_2 , suggesting a synergic effect between NaNO_2 and a protective culture. Results of this study were achieved based on experiments performed *in vitro* under laboratory conditions. For this reason, the inhibition effect cannot be extrapolated to fermented sausages because endogenous flora might have influence and interactions with the starter culture may occur. Despite these limitations, the *in vitro* experiments reported herein should help differentiate interactions between 2 bacterial species used as a starter culture with pathogenic flora. The Jameson effect hypothesis is supported in that suppression of a species over another can be attributed to competition for nutrients when nutrients are needed by both organisms. The interspecific competition parameter (β) < 1 indicated that prevailing competition in the co-culture was intraspecific with coexistence of bacterial species.

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