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Investigation of the selective bactericidal effect of several decontaminating solutions on bacterial biofilms including useful, spoilage and/or pathogenic bacteria

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Abstract

The aim of this work was to investigate the selective bactericidal effect of several decontaminating solutions on some spoilage, pathogenic and useful bacteria isolated from a traditional meat workshop.

Fourteen decontaminating solutions, i.e., acid, alkaline, osmotic, biocide solutions or their combinations were tested on five bacterial species grown as monospecies biofilms. The solution made of monolaurin (0.075% w/v) and acetic acid at pH 5.4 was the most selective decontaminating solution. It reduced by only 0.2 and 0.4 log *Lactobacillus* spp. and *Staphylococcus carnosus*, whereas *Pseudomonas fluorescens*, *Pseudomonas putida* and *Listeria monocytogenes* exhibited reductions of 3.7, 3.2 and 4.2 log, respectively. The acetic solution (pH 5.4) and the solution containing monolaurin (0.075% w/v) and sodium sulfate (12% w/v) were also selective. But their bactericidal effects on *Pseudomonas* species were relatively small.

Four selected solutions were then applied to seven bacterial species grown as multispecies biofilms. The mixture solution of monolaurin (0.075% w/v) and acetic acid at pH 5.4 showed again the best selectivity. Finally, lowering the pH of the acetic solution from 5.4 to 5.2 increased the selective decontamination.

Keywords: Selective decontamination; Biofilms; Meat; Useful bacteria; Spoilage bacteria; Pathogens

1. Introduction

Food products contamination may occur from environmental routes such as air, people and surfaces. The surface route is the most important to control on a day to day basis. This can be achieved by the implementation of a sanitation program (Holah, 1995). Disinfection is the final stage in a sanitation program. In addition to reducing the level of micro-organisms, it is designed to remove product residues and foreign bodies to ensure both the safety and quality of food products.

The manufacturers use harsh decontamination processes, killing most of the micro-organisms. For traditional fermented products, which are still often not inoculated with starter cultures and which depend on the indigenous flora, these harsh processes lead to significant losses of flavor and typicality of the products. The low sensorial properties of these products are due to the destruction of the useful flora. Thus, the setting up of targeted disinfection in workshops producing traditional fermented products is therefore necessary.

Few studies dealing with the selective decontamination have been recently reported. Promising results were obtained by Blaszyk and Holley (1998) who succeeded in inhibiting *Brochotrix thermosphacta* and *Listeria monocytogenes* with a solution containing sodium citrate, monolaurin and eugenol, while most of the lactic flora (*Lactobacillus sakei* and *Lactobacillus curvatus*) were preserved. In the same way, Vasseur (1999) observed that a combination of sodium sulfate (12%), sodium hydroxide (pH 10.5) and monolaurin or aliphatic fatty amine, allowed the destruction of *Listeria*

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monocytogenes and *Pseudomonas* species, without affecting the survival of some useful species like *Brevibacterium linens* and *Micrococcus* species. Although promising, these experiments were not carried out on multispecies biofilms, the common mode of bacterial attachment on surfaces.

The aim of the present study was to investigate the influence of pH, osmolarity and various molecules on monospecies biofilms formed by pathogenic, spoilage or useful bacteria in order to select those destroying the spoilage and pathogenic bacteria, while preserving the useful bacteria. In a second time, the selected solutions were tested on multispecies biofilms to verify their selectivity in the common mode of bacterial attachment.

2. Materials and methods

2.1. Micro-organisms

The bacterial strains used were isolated from a traditional meat workshop producing traditional dry sausages without inoculation of starter cultures to the batter (Chevallier et al., 2001). For the monospecies biofilm experiments, five micro-organisms were used: two useful bacteria (*Lactobacillus* spp. and *Staphylococcus carnosus*), two spoilage bacteria (*Pseudomonas fluorescens* and *Pseudomonas putida*) and one pathogenic bacterium (*Listeria monocytogenes*). For the multispecies biofilm experiments, *Hafnia alvei* and *Enterococcus faecium* were also included in order to have a better representation of spoilage species found in the traditional meat workshop (Chevallier et al., 2001).

2.2. Cultures

Bacteria isolates were precultivated and cultivated at 12°C (the temperature of the studied workshop) for 3–5 days in Standard Medium Broth (SMB) (AFNOR, NF ISO 4833: 1991) containing 5 g of tryptone (Biokar Diagnostics, Beauvais, France), 2.5 g of yeast extract (Beckton Dickinson, Le Pont de Claix, France) and 1 g of anhydrous glucose (Merck-eurolab, Briare Le Canal, France). To this medium, 2.5% (w/v) of NaCl (Merck-eurolab) was added in order to simulate the conditions of sausage manufacturing. After growing to approximately 10^6 cfu/ml (3–5 days according to each bacterium), cells were centrifuged at 12°C (MR 18.22, Jouan, France) for 10 min at 9000 *g*, washed 3 times in saline solution (physiologic water: 8 g/l of NaCl) and resuspended in 2 ml of saline solution.

2.3. Biofilm formation

Biofilms were grown according to the method of Tremoulet et al. (2002). A few modifications were carried out: Extra-thick fiberglass disks (diameter: 25 mm, Gelmann Sciences, MI, USA) were used as support matrix for biofilm growth. Disks were autoclaved at 121°C for 15 min prior to use. Two disks per culture were laid on Standard Medium Agar (SMA) (2.5% w/v of NaCl) for 1 h to allow their soaking with nutrients. Two other disks per culture were inoculated with 0.5 ml of bacterial culture at approximately 10^6 cfu/ml. After 5–10 min of contact allowing bacteria attachment, the inoculated disks were rinsed twice for 2 min with a saline water (20 ml contained in a Petri plate) under gentle agitation (45 rpm/s) (Orbital agitator 43 000, J.P. Selecta, France) to remove unattached cells. After rinsing, the inoculated disks were placed on the sterile disks already laid on the nutritive medium and incubation was run at 12°C for 5 days. One of the two inoculated disks was used as a control, the other was treated with the decontaminating solution.

For the multi-species biofilms experiments, the same protocol was used but disks inoculation was modified. Two disks were inoculated, each one, with a mixture (total: 581 µl) of 83 µl of each isolate culture at a concentration of approximately 10^6 cfu/ml. The inoculated disks were rinsed twice and placed on the two sterile disks already laid on the nutritive medium. The incubation was run at 12°C for 5 days. One of the two disks was used as a control and the other was treated with the decontaminating solution.

2.4. Decontaminating solutions and treatment procedures

The different decontaminating solutions were prepared in distilled water except monolaurin that was first solubilized in ethanol (95% v/v) (Merck-eurolab) and then diluted in distilled water. Fourteen decontaminating solutions composed of sodium hydroxide (Merck-eurolab), acetic acid (Merck-eurolab), sodium sulfate (Merck-eurolab), monolaurin (Sigma-Aldrich Chemie, Steinheim, Germany), quaternary ammonium (Bactégil, Sodevi, Saint-Etienne, France) and/or their combinations (Table 1) were tested on the monospecies biofilms. The most effective solutions were then applied on the multi-species biofilms to confirm their selectivity in the common mode of bacterial attachment.

The inoculated disks were introduced in a Petri dish containing 20 ml of the decontaminating solution for the appropriate time of exposure (Table 1) and under gentle agitation (60 rpm/s). Control and treated disks were rinsed twice (2 and 6 min) with sterile saline solution under gentle agitation (45 rpm/s) and placed in a stomacher bag (AES Laboratories, France) containing 20 ml of tryptone salt broth (Biokar Diagnostics). Then, they were stomached with a Lab Blender 400 stomacher (Seward, UK) for 6 min and decimal dilutions were pour plated onto the SMA plates (1% inoculum) using an automatic spiral plater WASP (Don Whitley Scientific

Limited, West Yorkshire, UK) and incubated at 12°C for 5 days.

For the multi-species biofilms, the different species were enumerated on selective media. The selective media used were MRS (De Man et al., 1960) for *Lactobacillus* spp., Chapman for *S. carnosus*, CFC for *Pseudomonas* species, Palcam for *L. monocytogenes*, Slanetz for *E. faecium* and VRBL for *H. alvei*. All these media were purchased from Biokar Diagnostics. Decimal dilutions of the obtained solutions were pour plated (1% inoculum) on the selective agar plates using the automatic agar plater and incubation were led at 12°C for 5 days.

The selectivity of each treatment was determined by comparing its bactericidal activity on the different species. The log-reduction was the difference between the log of initial cells counts and the log of survival cells following decontamination. Enumeration were performed using the WASP colony counting system. Experiments were performed in duplicate. Plates were incubated at 12°C in medium containing 2.5% (w/v) of NaCl and the decontaminating solutions were applied after 5 days of biofilms growth, i.e., conditions close to the manufacturing temperature and sanitation program used in the workshop.

Table 1
List of decontaminating solutions applied and their parameters

Treatment	Composition and parameters of the treatment
A	CH_3–COOH-pH 5.4 (30 min)
B	NaOH-pH 12 (30 min)
C	Na_2SO_4-12% w/v (pH 8.8) (30 min)
D	NaOH-pH 12 (30 min) + rinse + CH_3-COOH-pH 5.4 (30 min)
E	CH_3–COOH-pH 5.4 (30 min) + rinse + NaOH-pH 12 (30 min)
F	[NaOH-pH 12 + Na_2SO_4-12%] (pH = 11.3) (30 min)
G	[NaOH-pH 12 + monolaurine-0.075%)] (pH = 11.6) (30 min)
H	[NaOH-pH 12 + Na_2SO_4-12% (w/v) + monolaurine-0.075%] (pH = 11.5) (30 min)
I	Monolaurine-0.075% (w/v) (pH = 9.6) (30 min)
J	Bactégil[a] (Ammonium IV)-1% (v/v) (pH = 9) (5 min)
K	[CH_3–COOH + Na_2SO_4-12%] (pH = 5.4) (30 min)
L	[CH_3–COOH + monolaurine-0.075%] (pH = 5.4) (30 min)
M	[CH_3–COOH + Na_2SO_4-12% (w/v) + monolaurine-0.075%] (pH = 5.4) (30 min)
N	[Na_2SO_4-12% (w/v) + monolaurine-0.075%] (pH = 8.92) (30 min)
A″	CH_3–COOH-pH 5.2 (30 min)

[a]The disinfectant usually used in the studied traditional meat workshop.

2.5. Statistical analysis

The Statgraphics Plus program (Statistical Graphics Corp., Englewood Cliffs, NJ, USA) was used to carry out principal components analysis (PCA) on the obtained data.

3. Results and discussion

3.1. Bactericidal effects of decontaminating treatments on monospecies biofilms

The effect of the decontaminating solutions on bacteria is expressed in log-reductions of initial cell counts (Table 2).

Table 2
Log-reductions of bacterial cells grown in monospecies biofilms following exposition to decontaminating solutions

Treatment	Strains									
	Lactobacillus spp.		*Staphylococcus carnosus*		*Pseudomonas fluorescens*		*Pseudomonas putida*		*Listeria monocytogenes*	
	L.I.C	L.R	L.I.C	L.R	L.I.C	L.R	L.I.C	L.R	L.I.C	L.R
A	7.5	0.2	7.6	0.2	7.7	0.6	7.9	0.5	6.3	> 3.8
B	7.5	> 5.0	7.6	3.4	7.7	4.4	7.9	4.1	6.3	> 3.8
C	7.5	0.0	7.6	0.8	7.7	0.4	7.9	0.0	6.3	> 3.8
D	7.5	> 5.0	7.6	4.6	7.7	> 5.2	7.9	> 5.4	6.3	> 3.8
E	7.5	> 5.0	7.6	4.6	7.7	> 5.2	7.9	> 5.4	6.3	> 3.8
F	7.5	> 5.0	7.6	4.6	7.7	> 5.2	7.9	3.1	6.3	> 3.8
G	7.5	> 5.0	7.6	4.6	7.7	0.9	7.9	0.9	6.3	3.3
H	7.5	> 5.0	7.6	> 5.1	7.7	4.2	7.9	4.4	6.3	> 3.8
I	7.5	0.3	7.6	0.7	7.7	0.3	7.9	0.28	6.3	2.0
J	7.5	1.4	7.6	2.0	7.7	0.4	7.9	0.89	6.3	> 3.8
K	7.7	0.3	6.9	0.3	8.2	0.8	7.9	0.46	5.7	1.0
L	7.7	0.2	6.9	0.4	8.2	3.7	7.9	3.3	5.7	> 3.2
M	7.7	> 5.2	6.9	> 4.4	8.2	2.3	7.9	2.0	5.7	> 3.2
N	7.7	0.8	6.9	0.9	8.2	2.4	7.9	2.1	5.7	2.7

L.I.C: log of initial counts; L.R: log-reductions following treatments. See Table 1 for the composition of the decontaminating solutions.

3.1.1. Non-selective decontaminating solutions

The alkaline treatments containing NaOH (pH 12) alone (B) or in combination with other products (D, E, F, G, H) were not selective for the useful bacteria. Indeed, they were highly bactericidal since a large destruction was observed for most of the studied bacteria. More than 5 log-reductions were observed for the *Lactobacillus* strain. These results agree with Bourgeois et al. (1996) who showed that a pH higher than 11 was lethal for most of bacteria. Vasseur et al. (2001) also observed a complete destruction of *L. monocytogenes* and *Pseudomonas* species in solution of NaOH may be attributed to the dissolution of the polymeric substances involved in the binding of cells to surfaces (Vasseur, 1999). Another hypothesis results from the capacity of the alkaline solution to diffuse throughout the protective glycocalyx, to penetrate the cell wall, to disrupt the cytoplasmic membrane and to disturb the ionic gradients (Vasseur et al., 2001). The fast intracellular pH increase induces a saponification of the membrane's lipids and an inhibition of the membrane's energy producing ability. As a consequence, the energy production decreases and the bacterial growth is stopped (Vasseur et al., 2001).

The biocide treatment made of monolaurin (I) was also not selective since it had a weak inhibitory effect towards all the studied bacteria. These results agree with Vasseur et al. (2001) who showed that this biocide had a weak inhibitory activity towards *Pseudomonas* species. Oh and Marshall (1993, 1996) and Verhaegh et al. (1996) also showed that this biocide was not deleterious for *L. monocytogenes*.

Finally, the quaternary ammonium-'Bactegil' (J) was shown to be ineffective on the Gram-negative flora. But it showed an inhibitory effect on the Gram-positive bacteria mainly against the useful flora, even after 5 min of treatment. These results are in agreement with data reported by Isoard (1988) showing that quaternary ammoniums are ineffective on Gram-negative and active on Gram-positive bacteria.

3.1.2. Partially selective decontaminating solutions

The acetic acid solution (A) was partially selective. Indeed, the useful flora was less inhibited than the spoilage and pathogenic bacteria. Although it was efficient against the studied *L. monocytogenes* strain (more than 3.8 log), it had only a weak inhibitory effect against the spoilage bacteria (0.6 log). Results obtained with this treatment against *L. monocytogenes* do not agree with Buchanan et al. (1993) who reported that this bacterium was able to grow in HCl solution, pH 4.3. Three hypotheses may explain this difference. The first is related to the used acetic acid which has been reported to be more detrimental for bacteria than other organic or mineral acids (Vasseur et al., 1999). The second one is related to the "strain" factor. Ralovich (1992) reported that the minimum pH of growth for this bacterium depends on the studied strain. The last one is based on the fact that cells of the studied bacterium could be in autolysis phase regarding the period of incubation preceding the application of treatments and the amounts of NaCl added to the growth medium. Alternatively, the selectivity of acidic solutions for the useful flora agrees with the literature. Bourgeois et al. (1996) noted that staphylococci could grow at pH 4.2. Lactobacilli were also shown to be able to grow at pH of 3.3 (Kandler and Weiss, 1986; Kleynmans et al., 1989).

The osmotic treatment (C) made of sodium sulfate solution (12% w/v) was also partially selective. However it is less interesting than the acetic acid solution (A) since the useful flora, but also *Pseudomonas* species, were practically unaffected. These results are in agreement with several reports regarding the high resistance of *Pseudomonas* spp. (Vasseur et al., 2001), staphylococci (Kunin and Rudy, 1991) and lactic acid bacteria (Entani et al., 1986; Kandler and Weiss, 1986; Yanagida et al., 1987) to the osmotic stress. However, we showed that *L. monocytogenes* was largely affected by this treatment. This result does not agree with the observation of Arizcun et al. (1998) who reported that *L. monocytogenes* in biofilms are not very sensitive to osmotic stress (10.5% of NaCl). The influence of the "strain" factor or the fact that cells (see above) were in autolysis phase may explain the obtained results. Considering the "strain" factor, it has been shown that *Ps. fluorescens* is more sensitive to osmotic treatment than *P. putida* and *P. fragi* (Vasseur, 1999).

The treatment (K) combining the acetic acid solution (pH 5.4) and Na_2SO_4-12% (w/v) was also partially selective. Although the studied spoilage and pathogenic bacteria were much more inhibited than the useful bacteria, log-reduction levels were lower than or equal to 1.8 log. These levels cannot be sufficient to ensure the product safety. Our observations confirm the results of Arizcun et al. (1998) suggesting that sodium chloride and acid do not seem to have important effects in inactivating *L. monocytogenes*.

3.1.3. Selective decontaminating solutions

The solution (L) combining acetic acid solution (pH 5.4) and monolaurin (0.075% w/v) was selective since the studied spoilage and pathogenic micro-organisms, but not the useful bacteria, were largely inhibited (Table 2). As log-reductions of 3.7, 3.3 and more than 3.2 were observed for *P. fluorescens*, *P. Putida* and *L. monocytogenes*, respectively, *Lactobacillus* spp. and *S. carnosus* exhibited reductions of 0.2 and 0.4 log, respectively. These results agree with Oh and Marshall (1996) who showed a complete inactivation of 10^7 cfu/ml of *L. monocytogenes* by the synergetic action of 1% (v/v) acetic acid solution with 50 or 100 μg/ml of

monolaurin within 20 or 25 min. Wang and Johnson (1997) also observed that bactericidal activity of monolaurin was higher in acidic medium.

The solution made of sodium sulfate and monolaurin (N) was also selective since the spoilage and pathogenic bacteria were much more affected than the useful ones (Table 2). However, it was less efficient than the solution (L). The log-reduction levels did not exceed 2.7 log. The results obtained for solutions (I) and (N) confirmed the observations of Marshall (1998) who reported that antimicrobial effects of monolaurin increased with the decreasing of the water activity (a_w).

3.2. Principal components analysis of solution bactericidal effects

Considering the number of decontaminating solutions applied and the results against each species, it becomes difficult to have a global view of the whole results. Multivariate analysis technique such as principal components analysis (PCA) makes it possible to extract information related to bacteria physiologic responses to decontaminating solutions and to represent the results as a similarity map. PCA combines the variables that explain the largest part of the variance to form the first principal component. The second principal component accounts for the next largest part of variance, and so on, until the total sample variance is combined into component groups. PCA was applied to the log-reduction results obtained with the investigated treatments. The first two principal components (PC) accounted for 89.7% of the total variance with a predominance of the principal component 1 (47.6%) (Fig. 1). According to the PC1, the solutions A, C, I, J, K, L and N exhibited a positive score, whereas the B, D, E, F, G, H and M solutions displayed a negative score. These results are in agreement with the observations and it indicate that the treatments with a negative-score are very harmful to the useful bacteria. In addition, the treatment (L) and (N) were found on the far right of the map indicating that *L. monocytogenes* and *Pseudomonas* species, but not *Lactobacillus* spp. and *S. carnosus*, are highly inhibited by these two solutions.

Considering information extracted from the data and the PCA performed on the data, it was concluded that the solution made of acetic acid pH 5.4 and of monolaurin (0.075%) (L) was selective preserving the useful flora. The combination of monolaurin (0.075%) and sodium sulfate (12%) (N) was also selective for the useful flora but at a lesser extend than the solution (L). Although the acetic treatment (pH 5.4) was less effective on spoilage bacteria, it had a significant effect on *L. monocytogenes* and preserved efficiently the useful bacteria. Consequently, the treatments (A), (N) and (L) were selected for testing on multi-species biofilms and an acetic acid solution, pH 5.2 was also considered as a treatment.

3.3. Bactericidal effects of the selected treatments on multispecies biofilms

Table 3 shows the inactivation levels of the selected treatments on each bacterium of the multi-species biofilms. The results are expressed in log-reductions of initial cell counts.

The data obtained with the two acetic acid solutions (pH 5.4 and 5.2) (A and A″) confirmed the results obtained on the monospecies biofilms. Indeed, spoilage

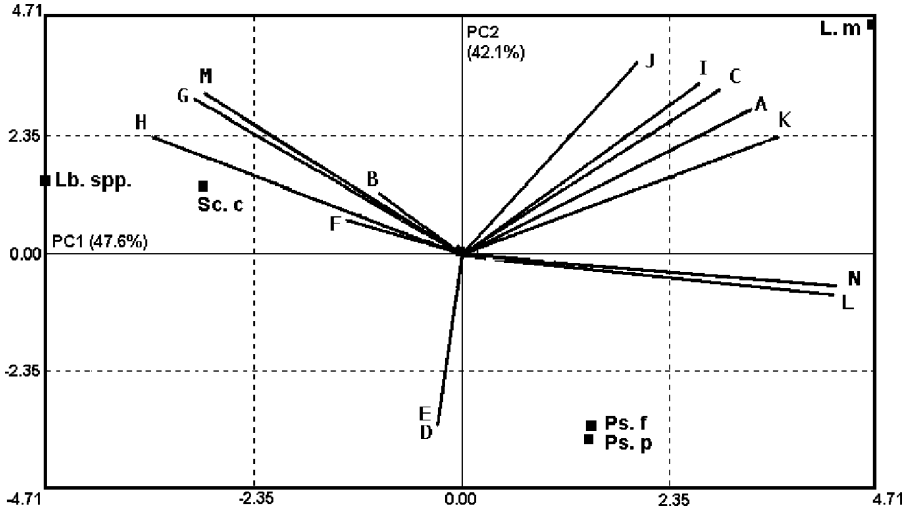

Fig. 1. Factorial biplot defined by the principal components 1 and 2 (PC 1: 47.6%; PC 2: 42.1%) resulting from the PCA performed on the log-reductions levels of monospecies biofilms. (See Table 1 for the correspondence with decontaminating solutions; Lb. spp.: *Lactobacillus* spp., L. m: *Listeria monocytogenes*, Ps. f: *Pseudomonas fluorescens*, Ps. p: *Pseudomonas putida*, Sc. C: *Staphylococcus carnosus*.)

and pathogenic bacteria were much more sensitive to the acidic treatments than the studied useful bacteria. Reductions of the harmful bacteria varied between 0.8 and 2.8 log, while the studied useful bacteria exhibited less than 0.2 log of reduction. Moreover we noted a stronger resistance of *L. monocytogenes* towards the acetic acid solution (pH 5.4) when grown in multi-species biofilms. Indeed, the log-reduction decreased from more than 3.8 in monospecies to 1.0 in multi-species. Inter-species communication may enhance the exchange of resistance genes to this stress. In fact, several authors reported the presence of soluble signals, such as autoinducer-2, that may be exchanged within multi-species communities to convey information between organisms (Miller and Bassler, 2001; Schauder and Bassler, 2001). Otherwise, we observed that decreasing the pH from 5.4 to 5.2 increased the selectivity of the acetic acid solution. The log-reductions of the useful bacteria remained practically constant, while those of the harmful bacteria increased considerably. A more concentrated acetic acid solution at pH between 4 and 5 is thought to be a very selective decontaminating solution.

Table 3
Log-reductions of bacterial cells grown in multispecies biofilms, following exposition to decontaminating solutions

Strains		Treatment			
		A	A″	L	N
Lactobacillus spp.	L.I.C	6.0	6.0	6.0	6.0
	L.R	0.1	0.2	0.1	0.1
Staphylococcus carnosus	L.I.C	4.1	4.1	4.1	4.1
	L.R	0.3	0.3	0.3	0.4
Enterococcus faecium	L.I.C	6.6	6.6	6.6	6.6
	L.R	1.3	1.4	3.8	1.1
Pseudomonas fluorescens	L.I.C	6.8	6.8	6.8	6.8
	L.R	2.2	2.8	4.2	1.6
Pseudomonas putida	L.I.C	6.7	6.7	6.7	6.7
	L.R	0.9	1.1	3.9	0.7
Hafnia alvei	L.I.C	6.9	6.9	6.9	6.9
	L.R	1.0	1.2	3.3	0.6
Listeria monocytogenes	L.I.C	6.2	6.2	6.2	6.2
	L.R	1.1	1.2	>3.7	0.6

L.I.C: log of initial counts; L.R: log-reductions following treatments. See Table 1 for the composition of the decontaminating solutions.

The combination of the acetic acid solution (pH 5.4) and monolaurin (0.075%) (L) was found to be very selective. The studied spoilage and pathogenic micro-organisms were largely affected by this treatment, whereas the useful bacteria were practically unaffected by the solution (L). Indeed, log-reductions varying between 3.3 and more than 3.7 were measured for the pathogenic and spoilage bacteria, whereas *Lactobacillus* spp. and *S. carnosus* exhibited only 0.1 and 0.3 log of reductions, respectively. These results confirmed the tendency observed for bacteria grown as monospecies biofilm and confirmed the synergetic action of monolaurin and acetic acid.

The solution containing sodium sulfate and monolaurin (N) was also selective since the spoilage and pathogenic bacteria were more inhibited than the useful bacteria (Table 3). However, the log-reduction levels remained insufficient to ensure the safety of the product. Moreover, the harmful bacteria were more resistant when grown in multi-species biofilms. The inter-species communication and the possibility of soluble signal exchanges could explain this observation (Miller and Bassler, 2001; Schauder and Bassler, 2001).

PCA was applied to the log-reduction result data obtained on multispecies biofilms. The two principal components (PC) accounted for 99.5% of the total variance with a large predominance of the principal

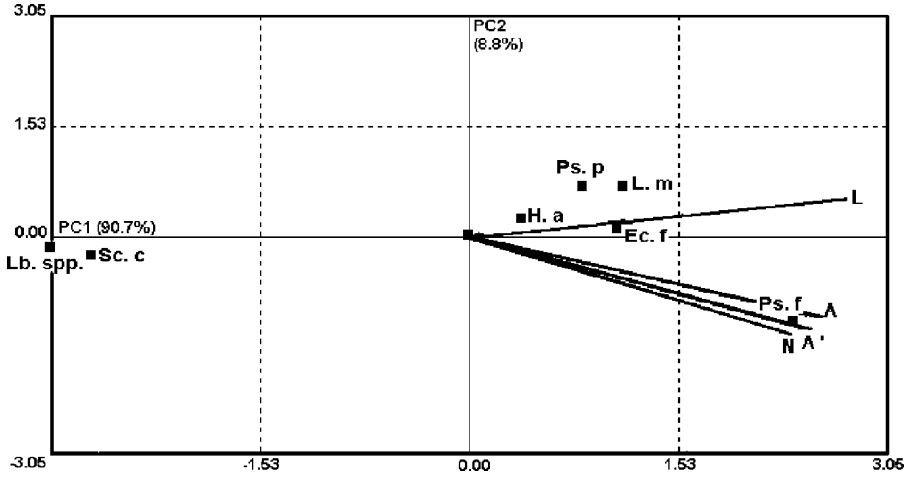

Fig. 2. Factorial biplot defined by the principal components 1 and 2 (PC 1: 90.7%; PC 2: 8.8%) resulting from the PCA performed on the log-reductions levels of multi-species biofilms. (See Table 1 for the correspondence with decontaminating solutions; Lb. spp.: *Lactobacillus* spp., Ec. f: *Enterococcus faecium*, H. a: *Hafnia alvei*, L. m: *Listeria monocytogenes*, Ps. f: *Pseudomonas fluorescens*, Ps. p: *Pseudomonas putida*, Sc. C: *Staphylococcus carnosus*.)

component 1 (PC1) (90.7%) (Fig. 2). It appeared that the four decontaminating solutions (A, A″, L, N) exhibited a positive score. This result was explained by the fact that all these treatments were not harmful to the useful bacteria. Thus, the PC1 discriminated the useful bacteria from the pathogenic and spoilage ones. Likewise, the PC2 discriminated the four treatments into two groups. The first group is made of the treatment (L), which was harmful to the pathogenic and spoilage bacteria. The other group contained the treatment (A, A″ and N) which was less effective against the harmful bacteria and had a preservative effect on useful bacteria. These observations confirm again the highest selectivity of the treatment (L).

4. Conclusion

We can conclude that monolaurin and acetic acid have a synergetic and selective actions, preserving the useful bacteria and inactivating the pathogenic and spoilage bacteria. The acetic acid solution pH 5.4 could be a targeting disinfectant, but it is insufficiently efficient. Lowering the pH of this solution to pH 5.2 increased its efficiency. Alternatively, *L. monocytogenes* is more resistant when grown in multi-species biofilm than when grown as monospecies biofilm. But the synergetic effect of monolaurin and acetic acid enhanced the inactivation of *L. monocytogenes*. The obtained results suggest that selective decontamination processes can be set up in traditional meat workshops allowing to preserve the useful flora and as a consequence the typicality of the dry sausages.

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