Lactic Acid Bacteria Isolated from Chicken Carcasses with Inhibitory Activity against *Salmonella* spp. and *Listeria monocytogenes*

Ioannis SAKARIDIS[1], Nikolaos SOULTOS[1], Christos BATZIOS[2], Ioannis AMBROSIADIS[1] and Pavlos KOIDIS[1]

[1]Department of Hygiene and Technology of Foods of Animal Origin and [2]Laboratory of Animal Production Economics, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Abstract

Sakaridis I., Soultos N., Batzios Ch., Ambrosiadis I., Koidis P. (2014): **Lactic acid bacteria isolated from chicken carcasses with inhibitory activity against *Salmonella* spp. and *Listeria monocytogenes***. Czech J. Food Sci., **32**: 61–68.

Lactic acid bacteria (LAB) isolated from poultry carcasses were added to BHI broth along with *Salmonella* spp. and *Listeria monocytogenes* in order to determine their antagonistic activity against the pathogens. There was a statistically significant reduction in *Salmonella* population on the 5th day that varied from 0.41 to 1.12 log CFU/ml. The reduction in *L. monocytogenes* population was also statistically significant and varied from 0.77 to 1.48 log CFU/ml. The LAB strain with the best inhibitory activity was chosen to examine its action against the same pathogens on the chicken skin and meat. On the chicken skin, the growth reduction on the 6th day caused by *L. salivarius* was lower and did not exceed the 0.54 log CFU/cm^2 for *Salmonella* spp. and 0.71 log CFU/cm^2 for *L. monocytogenes*. The reduction on the chicken meat was slightly lower for both pathogens. The results of the experiments suggest that *L. salivarius* (strain LAB 59) has a potential to be used as a protective culture to improve the safety and extend the shelf life of chicken products.

Keywords: biopreservation; poultry; *Salmonella* sp.; *Listeria* sp.

Poultry meat is considered as one of the most common foods that cause foodborne infection and intoxication. The consumption of broiler meat and products thereof was implicated in 28 verified (3.7%) outbreaks in the EU during 2008, with *Salmonella* spp., especially *S. enteritidis,* being the causative agent (EFSA 2010). The need of consumers to have a safe product with extended shelf life pushes the research towards this direction. Many chemical or physical decontamination treatments have been used or suggested such as chlorine, organic acids, phosphates, hydrogen peroxide, ozone, ultrahigh hydrostatic pressure, irradiation, pulsed-field electricity, ultrasonic energy, UV light, and others (Loretz *et al*. 2010). However, consumers tend to prefer natural ways for improving the safety of poultry products and controlling undesirable microorganisms without altering their desirable characteristics. In this respect, biopreservation has gained increased attention as a means of naturally controlling the shelf life and safety of food products. This biopreservation approach refers to the extended storage life and enhanced safety of food using their natural or controlled microflora and their antibacterial products (Holzapfel *et al*. 1995). These protective cultures are antagonistic cultures added to meat products to inhibit pathogens and/or prolong the shelf life, while changing the sensory properties as little as possible (Lucke 2000). Lactic acid bacteria (LAB) constitute the most appropriate candidate for the application as protective cultures since they are present in all fermented foods, have a long history of safe use, and form part of the gut microflora of humans and animals (Maragkoudakis *et al*. 2009).

Biopreservation is based on microbial antagonism or microbial interference. This refers to the inhibition of undesirable or pathogenic microorganisms, caused by the competition for nutrients and by the production of antimicrobial metabolites such as organic acids, hydrogen peroxide, diacetyl, reuterin, bacteriocins, and other low molecular weight metabolites (Holzapfel 1995; Jay 1996).

Over the last few decades, LAB have been extensively used for preserving fermented and cooked meat products and a variety of strains have been found to be effective against pathogens and spoilage organisms related to those products (Vermeiren *et al*. 2003; Kostrzynska & Bachard 2006; Leroy *et al*. 2006). However, the investigation into biopreservation of fresh red meat is rather limited (Muthukumarasamy *et al*. 2003; Senne & Gilliland 2003; Laursen *et al*. 2005) and even scarcer into that of fresh poultry meat (Brashears *et al*. 1998; Maragkoudakis *et al*. 2009). To the best of our knowledge, stock cultures of LAB have been used to check their antagonistic action towards pathogens on raw poultry; on the contrary, LAB isolated from the natural microflora of the skin of poultry carcasses have not been used for the biopreservation of raw poultry products so far.

The objective of this study was to evaluate the antimicrobial potential of seven selected strains of lactic acid bacteria isolated from poultry carcasses using the double layer inhibition method described by Sakaridis *et al*. (2012). During their isolation, these strains showed an initial antagonistic activity against pathogenic bacteria like *Salmonella* spp. and *Listeria monocytogenes* that were also isolated from poultry carcasses in previous studies (Sakaridis *et al*. 2011a,b). However, it was a challenge to investigate their antagonistic activity against *Salmonella* spp. and *Listeria monocytogenes in vivo* and to evaluate their potential for practical application. This interaction will take place on three different media; in broths, on chicken skin, and on chicken meat.

MATERIAL AND METHODS

Preparation of pathogens and lactic acid bacteria cultures. A cocktail of six strains of *Salmonella* (*S. blockley, S. paratyphi* B, *S. bredeney, S. neftenbach, S. hadar*, and *S. thompson*) and another cocktail of six strains of *Listeria monocytogenes* were used. Both *Salmonella* and *Listeria monocytogenes* strains were isolated from poultry carcasses in previous studies (Sakaridis *et al*. 2011a,b). These strains were cultivated at 37°C for 48 h in Brain-Heart Infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) before being diluted to previously determined concentrations (10^6 CFU/ml). A 2 ml volume of each culture was pooled to provide the two cocktails.

Lactic acid bacteria isolated from poultry carcasses in a previous study (Sakaridis *et al*. 2012) that exhibited antibacterial activity using the double layer inhibition method against these strains of *Salmonella* spp. and *Listeria monocytogenes* were also selected. These seven strains were found to belong to the species of *Lactobacillus johnsonii* (LAB 5), *Lactobacillus salivarius* (LAB 40 and 59), *Lactobacillus paralimentarius* (LAB 51), *Lactobacillus reuteri* (LAB 74), and *Pediococcus acidilactici* (LAB 7 and 48). For each experiment, they were cultivated at 20°C for 72 h in MRS broth (Merck KGaA, Darmstadt, Germany) before being diluted to previously determined concentrations (10^8 CFU/ml).

Fresh BHI cultures of *Salmonella* spp. and *Listeria monocytogenes* isolates and fresh MRS cultures of lactic acid bacteria were prepared the day before each experiment.

Antagonistic activity in broths. For evaluating the antagonistic activity of the LAB strains, 1 ml of each LAB culture (10^8 CFU/ml) and 1 ml of the pathogens cultures (10^6 CFU/ml) were added to 8 ml of sterile BHI broth so as to make their final concentrations 10^7 CFU/ml and 10^5 CFU/ml, respectively. One ml of LAB culture, *Salmonella* spp. culture, and *Listeria monocytogenes* culture were also added to 9 ml of BHI broth to monitor their populations during the experiment. All samples were kept at 7°C and were subjected to microbial analysis on days 0, 1, 2, 3, 4, and 5.

Antagonistic activity on chicken skin. Breast skin samples (of approximately 40 cm^2) from poultry carcasses straight after the slaughtering process were collected and placed on sterile Petri dishes. The samples were transported to the laboratory within an hour after collection in coolers with ice and were processed immediately. Each skin sample was cut using a sterile knife into four pieces of 10 cm^2 and sanitised by exposure to a germicidal UV light G30T8 254 nm (Philips Ultra Violet, Amsterdam, the Netherlands), at a distance of 40–45 cm for 15 min for one side and another 15 min for the other side of the skin. The UV lamp was turned on 1 h prior to each experiment. 0.1 ml of the LAB culture (10^8 CFU/ml) with the best inhibitory activity found from the broth experiment and 0.1 ml of *Salmonella* (10^6 CFU/ml) and/or *Listeria monocytogenes* culture (10^6 CFU/ml) were placed on the geometrical centre and spread

thoroughly on the surface of each skin sample, the final concentrations being 10^6 CFU/cm^2 for the LAB and 10^4 CFU/cm^2 for the pathogens. Skin samples inoculated only with the LAB strain, or *Salmonella*, or *Listeria monocytogenes* were also prepared simultaneously with those that were kept intact. UV treated skin samples were also analysed to evaluate the effectiveness of the UV treatment. All samples were kept at 7°C and were subjected to microbial analysis on days 0, 1, 2, 3, 4, 5, and 6.

Antagonistic activity on chicken meat. Breast meat samples from poultry carcasses right after the slaughtering process were collected and placed on sterile stomacher bags. The samples were transported to the laboratory within an hour after collection in coolers with ice and were processed immediately. Breast meat was aseptically cut into pieces of 10 cm^2 and 5 mm depth using a sterile knife and cutting board. 0.1 ml of the LAB culture (10^8 CFU/ml) with the best inhibitory activity found from the previous experiment and 0.1 ml of *Salmonella* (10^6 CFU/ml) and/or *Listeria monocytogenes* culture (10^6 CFU/ml) were placed on the geometrical centre and spread thoroughly on the surface of each meat sample, the final concentrations being 10^6 CFU/cm^2 for the LAB and 10^4 CFU/cm^2 for the pathogens. Meat samples inoculated only with the LAB strain, or *Salmonella*, or *Listeria monocytogenes* were also prepared simultaneously with those that were kept intact. All samples were kept at 7°C and were subjected to microbial analysis on days 0, 1, 2, 3, 4, 5, and 6.

Microbial analyses. For all experiments, the samples were diluted using sterile peptone (0.1%) dilution blanks and plated using the spread plate technique. The initial dilutions for the chicken skin and meat samples were prepared by adding diluent to each sample to prepare the 1:10 dilution and were blended for 1 min in a laboratory blender Stomacher 400 (Seward Medical, London, UK). Appropriate additional decimal dilutions in sterile peptone water (0.1%) were plated in duplicates on the selective media to enumerate the inoculated microorganisms. XLD agar (Merck KGaA, Darmstadt, Germany) was used to enumerate *Salmonella* and the plates were incubated at 37°C for 48 hours. Agar Listeria Ottavani and Agosti medium (ALOA; Biolife, Milan, Italy) was used to enumerate *Listeria monocytogenes*; the plates of this medium were incubated at 37°C for 48 hours. Finally, MRS agar (Merck KGaA, Darmstadt, Germany) was used to enumerate lactic acid bacteria. The plates were kept at 30°C for 48 h under anaerobic conditions. The mean value of the two Petri dishes was taken into account and all experiments were carried out in triplicates.

Sensory evaluation. Sensory evaluation of the control and the samples inoculated with the LAB strains was conducted in the open laboratory. Odour and appearance of slime on the external surface of the chicken skin and meat were assessed.

Statistical analysis. For the statistical analysis and evaluation of the experimental data, both parametric and nonparametric statistical methods were applied. As all forms of parametric tests are based on the assumption that the within-groups data are samples drawn from normally distributed populations with equal variances, both formal tests (Shapiro-Wilk and Lilliefors tests) and graphical displays were performed for assessing the departures from normality, while the variances were tested for homogeneity using the Levene's test. For accessing the assumptions of normality and stability of variances, the data were also transformed to $\log_e$, $\log_{10}$, or sqrt (Zolman 1993).

More particularly, in the case of normality and variances homogeneity, one way analysis of variance (One-way ANOVA) was performed, to evaluate possible significant effects of the treatment on the population of *Listeria monocytogenes* and of *Salmonella* spp. in BHI broths. The differences between the mean values of specific treatments and for specific days of storage were evaluated using the Duncan's new multiple range test. Where the assumptions about either variability or the form of the populations distribution were seriously violated, with or no transformed data, the Kruskal-Wallis nonparametric test was applied to evaluate the treatment depended differences, while the differences between the mean values of the specific treatments were evaluated using the nonparametric Wilcoxon rank sum test (Mann-Whitney *U*-test).

Regarding the population of *Listeria monocytogenes* and of *Salmonella* spp. on chicken meat and on chicken skin, *t*-test was used to evaluate the differences between the treatments in the case of normality, while the Wilcoxon rank sum nonparametric test was applied where the assumptions about the form of the populations distribution were seriously violated.

All analyses were conducted using the statistical software program SPSS for Windows v. 15.0. The significance was declared at $P \leq 0.05$, unless otherwise noted.

RESULTS

Antagonistic activity in broths. The seven selected strains of LAB (LAB 5 – *Lactobacillus johnsonii*, LAB 7 and 48 – *Pediococcus acidilactici*, LAB 40 and 59 – *Lactobacillus salivarius*, LAB 51 – *Lactoba-*

Table 1. Inhibition of pathogens growth in broths by LAB (log_{10}, mean ± SD)

	Time (day)	*Salmonella* (CFU/ml)		*Listeria* (CFU/ml)	
		log_{10}	mean ± SD	Log_{10}	mean ± SD
Pathogen	1 = 0	5.00	$10 \times 10^{4\,a} \pm 1.41 \times 10^4$	5.00	$10 \times 10^{4\,a} \pm 1.41 \times 10^4$
	2	5.84	$70.16 \times 10^{4\,a} \pm 3.49 \times 10^4$	5.21	$16.33 \times 10^{4\,a} \pm 3.08 \times 10^4$
	3	6.04	$110.33 \times 10^{4\,a} \pm 8.36 \times 10^4$	5.91	$82.33 \times 10^{4\,a} \pm 9.46 \times 10^4$
	4	6.17	$148.5 \times 10^{4\,a} \pm 8.67 \times 10^4$	6.33	$201 \times 10^{4\,a} \pm 19.34 \times 10^4$
	5	6.32	$208 \times 10^{4\,a} \pm 6.42 \times 10^4$	6.40	$250.33 \times 10^{4\,a} \pm 12.97 \times 10^4$
	6	6.48	$302.33 \times 10^{4\,a} \pm 14.70 \times 10^4$	6.49	$307 \times 10^{4\,a} \pm 21.82 \times 10^4$
Pathogen + LAB 5	1 = 0	4.99	$9.83 \times 10^{4\,a} \pm 1.17 \times 10^4$	5.01	$10.33 \times 10^{4\,a} \pm 1.63 \times 10^4$
	2	5.16	$14 \times 10^{4\,e} \pm 1.90 \times 10^4$	5.07	$11.83 \times 10^{4\,bc} \pm 1.72 \times 10^4$
	3	5.48	$30.17 \times 10^{4\,f} \pm 3.43 \times 10^4$	5.06	$11.5 \times 10^{4\,bc} \pm 1.87 \times 10^4$
	4	5.59	$38.67 \times 10^{4\,e} \pm 5.28 \times 10^4$	5.17	$14.83 \times 10^{4\,bc} \pm 2.56 \times 10^4$
	5	5.79	$61.67 \times 10^{4\,c} \pm 6.12 \times 10^4$	5.23	$17 \times 10^{4\,c} \pm 3.90 \times 10^4$
	6	5.97	$93.83 \times 10^{4\,d} \pm 6.58 \times 10^4$	5.49	$31 \times 10^{4\,c} \pm 4.56 \times 10^4$
Pathogen + LAB 7	1 = 0	5.01	$10.33 \times 10^{4\,a} \pm 2.25 \times 10^4$	5.01	$10.17 \times 10^{4\,a} \pm 1.47 \times 10^4$
	2	5.26	$18.33 \times 10^{4\,d} \pm 3.39 \times 10^4$	5.11	$13 \times 10^{4\,b} \pm 2.19 \times 10^4$
	3	5.59	$39.33 \times 10^{4\,e} \pm 4.50 \times 10^4$	5.08	$12.17 \times 10^{4\,b} \pm 1.94 \times 10^4$
	4	5.91	$81.83 \times 10^{4\,c} \pm 8.52 \times 10^4$	5.14	$13.83 \times 10^{4\,cd} \pm 2.71 \times 10^4$
	5	6.00	$101 \times 10^{4\,b} \pm 10.37 \times 10^4$	5.12	$13.17 \times 10^{4\,de} \pm 1.72 \times 10^4$
	6	6.04	$110.5 \times 10^{4\,e} \pm 11.29 \times 10^4$	5.39	$24.83 \times 10^{4\,d} \pm 4.17 \times 10^4$
Pathogen + LAB 40	1 = 0	5.00	$10 \times 10^{4\,a} \pm 2.10 \times 10^4$	4.99	$9.83 \times 10^{4\,a} \pm 1.17 \times 10^4$
	2	5.21	$16.17 \times 10^{4\,de} \pm 1.72 \times 10^4$	5.00	$10 \times 10^{4\,c} \pm 1.41 \times 10^4$
	3	5.71	$51.83 \times 10^{4\,d} \pm 5.31 \times 10^4$	4.99	$9.83 \times 10^{4\,c} \pm 2.32 \times 10^4$
	4	5.78	$60.83 \times 10^{4\,d} \pm 7.57 \times 10^4$	5.09	$12.33 \times 10^{4\,d} \pm 1.75 \times 10^4$
	5	5.83	$67.17 \times 10^{4\,c} \pm 4.17 \times 10^4$	5.05	$11.33 \times 10^{4\,ef} \pm 2.25 \times 10^4$
	6	5.85	$71.50 \times 10^{4\,c} \pm 4.46 \times 10^4$	5.31	$20.33 \times 10^{4\,e} \pm 1.63 \times 10^4$
Pathogen + LAB 48	1 = 0	5.01	$10.33 \times 10^{4\,a} \pm 1.36 \times 10^4$	4.99	$9.83 \times 10^{4\,a} \pm 1.33 \times 10^4$
	2	5.48	$30.33 \times 10^{4\,c} \pm 4.46 \times 10^4$	5.01	$10.17 \times 10^{4\,c} \pm 1.94 \times 10^4$
	3	5.86	$72.17 \times 10^{4\,c} \pm 4.49 \times 10^4$	5.11	$13 \times 10^{4\,b} \pm 1.79 \times 10^4$
	4	5.94	$86.5 \times 10^{4\,c} \pm 7.71 \times 10^4$	5.21	$16.33 \times 10^{4\,b} \pm 1.63 \times 10^4$
	5	5.99	$98.83 \times 10^{4\,b} \pm 4.96 \times 10^4$	5.17	$14.67 \times 10^{4\,cd} \pm 2.87 \times 10^4$
	6	6.04	$110.5 \times 10^{4\,e} \pm 5.89 \times 10^4$	5.41	$25.50 \times 10^{4\,d} \pm 4.32 \times 10^4$
Pathogen + LAB 51	1 = 0	5.00	$10 \times 10^{4\,a} \pm 1.41 \times 10^4$	5.00	$10 \times 10^{4\,a} \pm 1.41 \times 10^4$
	2	5.61	$40.5 \times 10^{4\,b} \pm 4.59 \times 10^4$	5.02	$10.5 \times 10^{4\,c} \pm 1.76 \times 10^4$
	3	5.91	$80.5 \times 10^{4\,b} \pm 7.53 \times 10^4$	5.06	$11.5 \times 10^{4\,bc} \pm 1.87 \times 10^4$
	4	5.99	$98 \times 10^{4\,b} \pm 4.38 \times 10^4$	5.16	$14.33 \times 10^{4\,bcd} \pm 1.86 \times 10^4$
	5	6.02	$104.17 \times 10^{4\,b} \pm 10.13 \times 10^4$	5.39	$24.67 \times 10^{4\,b} \pm 3.01 \times 10^4$
	6	6.07	$118.5 \times 10^{4\,e} \pm 8.67 \times 10^4$	5.72	$52.5 \times 10^{4\,b} \pm 5.86 \times 10^4$
Pathogen + LAB 59	1 = 0	5.00	$10 \times 10^{4\,a} \pm 1.09 \times 10^4$	4.98	$9.67 \times 10^{4\,a} \pm 0.82 \times 10^4$
	2	5.03	$10.83 \times 10^{4\,f} \pm 1.17 \times 10^4$	5.00	$10 \times 10^{4\,c} \pm 1.41 \times 10^4$
	3	5.19	$15.67 \times 10^{4\,g} \pm 2.34 \times 10^4$	4.99	$9.83 \times 10^{4\,c} \pm 1.47 \times 10^4$
	4	5.25	$17.83 \times 10^{4\,f} \pm 2.48 \times 10^4$	5.00	$10 \times 10^{4\,e} \pm 1.26 \times 10^4$
	5	5.30	$20 \times 10^{4\,d} \pm 2.45 \times 10^4$	5.01	$10.17 \times 10^{4\,f} \pm 1.60 \times 10^4$
	6	5.36	$22.83 \times 10^{4\,b} \pm 3.31 \times 10^4$	5.01	$10.33 \times 10^{4\,bf} \pm 1.75 \times 10^4$
Pathogen + LAB 74	1 = 0	4.99	$9.83 \times 10^{4\,a} \pm 1.47 \times 10^4$	5.01	$10.17 \times 10^{4\,a} \pm 1.17 \times 10^4$
	2	5.42	$26.5 \times 10^{4\,c} \pm 3.39 \times 10^4$	5.05	$11.33 \times 10^{4\,bc} \pm 1.75 \times 10^4$
	3	5.71	$51.5 \times 10^{4\,d} \pm 6.86 \times 10^4$	5.03	$10.83 \times 10^{4\,bc} \pm 1.47 \times 10^4$
	4	5.91	$81.83 \times 10^{4\,c} \pm 5.42 \times 10^4$	5.08	$12 \times 10^{4\,d} \pm 1.41 \times 10^4$
	5	5.99	$97.33 \times 10^{4\,b} \pm 5.46 \times 10^4$	5.19	$15.33 \times 10^{4\,cd} \pm 1.21 \times 10^4$
	6	6.04	$108.83 \times 10^{4\,e} \pm 8.42 \times 10^4$	5.39	$24.67 \times 10^{4\,d} \pm 3.98 \times 10^4$

[a–g]mean values in the same column and for the same time of storage with superscript in common do not differ significantly ($P > 0.05$)

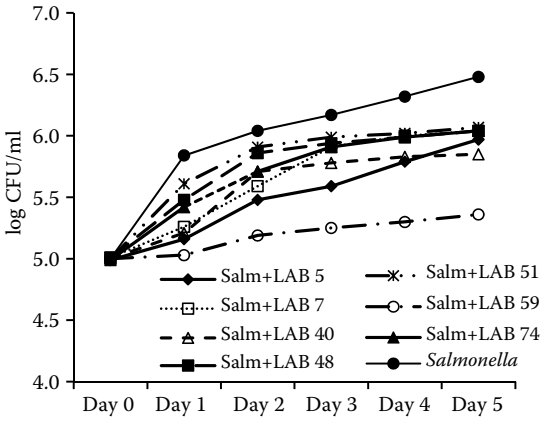

Figure 1. Growth of *Salmonella* spp. in the presence (LAB 5, 7, 40, 48, 51, 59, 74) or absence (*Salmonella*) of LAB in broths

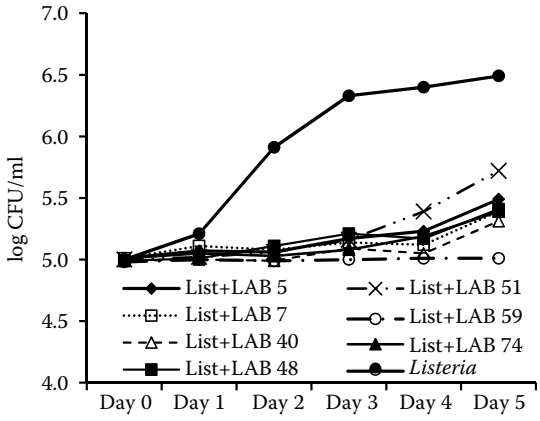

Figure 2. Growth of *Listeria monocytogenes* in the presence (LAB 5, 7, 40, 48, 51, 59, 74) or absence (*Listeria*) of LAB in broths

cillus paralimentarius, and LAB 74 – *Lactobacillus reuteri*) were tested in broths for their antagonistic properties against the pool of *Salmonella* spp. and *Listeria monocytogenes*. All LAB isolates survived well in the broths at the temperature of 7°C and their population levels remained constant throughout the 5 days storage with or without the presence of the pathogens. The growth of the pathogens, however, was adversely affected by the presence of the LAB isolates. There was a statistically significant reduction ($P \leq 0.05$) in *Salmonella* population (Salm) on the 5th day that varied from 0.41 to 1.12 log CFU/ml (Salm + LAB 5: 5.97 log CFU/ml, Salm + LAB 7: 6.04 log CFU/ml, Salm + LAB 40: 5.85 log CFU/ml, Salm + LAB 48: 6.04 log CFU/ml, Salm + LAB 51: 6.07 log CFU/ml, Salm + LAB 59: 5.36 log CFU/ml, Salm + LAB 74: 6.04 log CFU/ml, *Salmonella*: 6.48 log CFU/ml) compared to the broth inoculated only with *Salmonella* spp. (Table 1 or Figure 1).The protective effect of the LAB isolates was also statistically significant ($P \leq 0.05$) for *Listeria monocytogenes* (List) where the reduction of its population on the 5th day varied from 0.77 to 1.48 log CFU/ml (List + LAB 5: 5.49 log CFU/ml, List + LAB 7: 5.39 log CFU/ml, List + LAB 40: 5.31 log CFU/ml, List + LAB 48: 5.41 log CFU/ml, List + LAB 51: 5.72 log CFU/ml, List + LAB 59: 5.01 log CFU/ml, List + LAB 74: 5.39 log CFU/ml, *Listeria monocytogenes*: 6.49 log CFU/ml) compared to the broth inoculated only with *Listeria monocytogenes* (Table 1 or Figure 2).The statistical analysis of the results

Table 2. Inhibition of pathogens growth by *L. salivarius* on chicken skin

	Time (day)	*Salmonela* (CFU/cm^2)		*Listeria* (CFU/cm^2)	
		$\log_{10}$	mean ± SD	$\log_{10}$	mean ± SD
Pathogen	1 = 0	4.03	$1.07 \times 10^{4\,a} \pm 0.22 \times 10^4$	4.05	$1.12 \times 10^{4\,a} \pm 0.15 \times 10^4$
	2	4.21	$1.63 \times 10^{4\,a} \pm 0.25 \times 10^4$	4.19	$1.55 \times 10^{4\,a} \pm 0.29 \times 10^4$
	3	4.52	$3.30 \times 10^{4\,a} \pm 0.35 \times 10^4$	4.28	$1.90 \times 10^{4\,a} \pm 0.35 \times 10^4$
	4	4.93	$8.58 \times 10^{4\,a} \pm 0.52 \times 10^4$	4.94	$8.75 \times 10^{4\,a} \pm 0.39 \times 10^4$
	5	5.27	$18.48 \times 10^{4\,a} \pm 1.03 \times 10^4$	5.25	$17.75 \times 10^{4\,a} \pm 0.67 \times 10^4$
	6	5.42	$26.12 \times 10^{4\,a} \pm 0.94 \times 10^4$	5.37	$23.65 \times 10^{4\,a} \pm 0.92 \times 10^4$
	7	5.53	$34.17 \times 10^{4\,a} \pm 1.24 \times 10^4$	5.51	$32.43 \times 10^{4\,a} \pm 0.81 \times 10^4$
Pathogen + LAB 59	1 = 0	4.51	$1.07 \times 10^{4\,a} \pm 0.17 \times 10^4$	4.00	$1.00 \times 10^{4\,a} \pm 0.18 \times 10^4$
	2	4.03	$1.37 \times 10^{4\,a} \pm 0.16 \times 10^4$	4.16	$1.43 \times 10^{4\,a} \pm 0.17 \times 10^4$
	3	4.14	$2.10 \times 10^{4\,b} \pm 0.26 \times 10^4$	4.25	$1.80 \times 10^{4\,a} \pm 0.26 \times 10^4$
	4	4.32	$3.92 \times 10^{4\,b} \pm 0.59 \times 10^4$	4.46	$2.92 \times 10^{4\,b} \pm 0.35 \times 10^4$
	5	4.59	$6.02 \times 10^{4\,b} \pm 0.49 \times 10^4$	4.60	$4.02 \times 10^{4\,b} \pm 0.38 \times 10^4$
	6	4.78	$7.87 \times 10^{4\,b} \pm 0.49 \times 10^4$	4.70	$5.03 \times 10^{4\,b} \pm 0.60 \times 10^4$
	7	4.90	$9.93 \times 10^4 \pm 0.69 \times 10^4$	4.81	$6.40 \times 10^4 \pm 0.52 \times 10^4$

^{a,b}mean values in the same column and for the same time of storage with superscript in common do not differ significantly ($P > 0.05$)

Table 3. Inhibition of pathogens growth by *L. salivarius* on chicken meat

	Time (day)	*Salmonella* (CFU/cm^2)		*Listeria* (CFU/cm^2)	
		$\log_{10}$	mean ± SD	$\log_{10}$	mean ± SD
Pathogen	1 = 0	3.97	$0.93 \times 10^{4\,a} \pm 0.08 \times 10^4$	3.97	$0.93 \times 10^{4\,a} \pm 0.08 \times 10^4$
	2	4.19	$1.55 \times 10^{4\,a} \pm 0.27 \times 10^4$	4.20	$1.60 \times 10^{4\,a} \pm 0.25 \times 10^4$
	3	4.52	$3.32 \times 10^{4\,a} \pm 0.40 \times 10^4$	4.31	$2.07 \times 10^{4\,a} \pm 0.27 \times 10^4$
	4	4.91	$8.08 \times 10^{4\,a} \pm 0.65 \times 10^4$	4.93	$8.47 \times 10^{4\,a} \pm 0.46 \times 10^4$
	5	5.26	$18.18 \times 10^{4\,a} \pm 1.13 \times 10^4$	5.23	$16.95 \times 10^{4\,a} \pm 0.68 \times 10^4$
	6	5.41	$25.53 \times 10^{4\,a} \pm 1.56 \times 10^4$	5.37	$23.33 \times 10^{4\,a} \pm 0.67 \times 10^4$
	7	5.49	$30.93 \times 10^{4\,a} \pm 0.99 \times 10^4$	5.46	$28.90 \times 10^{4\,a} \pm 1.13 \times 10^4$
Pathogen + LAB 59	1 = 0	4.04	$1.10 \times 10^{4\,a} \pm 0.19 \times 10^4$	4.00	$1.00 \times 10^{4\,a} \pm 0.20 \times 10^4$
	2	4.08	$1.22 \times 10^{4\,b} \pm 0.15 \times 10^4$	4.20	$1.60 \times 10^{4\,a} \pm 0.24 \times 10^4$
	3	4.33	$2.13 \times 10^{4\,b} \pm 0.26 \times 10^4$	4.29	$1.97 \times 10^{4\,a} \pm 0.20 \times 10^4$
	4	4.61	$4.10 \times 10^{4\,b} \pm 0.48 \times 10^4$	4.49	$3.12 \times 10^{4\,b} \pm 0.33 \times 10^4$
	5	4.78	$6.08 \times 10^{4\,b} \pm 0.90 \times 10^4$	4.62	$4.13 \times 10^{4\,b} \pm 0.50 \times 10^4$
	6	4.91	$8.05 \times 10^{4\,b} \pm 0.74 \times 10^4$	4.71	$5.13 \times 10^{4\,b} \pm 0.33 \times 10^4$
	7	4.98	$9.58 \times 10^{4\,b} \pm 0.81 \times 10^4$	4.79	$6.15 \times 10^{4\,b} \pm 0.54 \times 10^4$

[a,b]mean values in the same column and for the same time of storage with superscript in common do not differ significantly ($P > 0.05$)

from the broths with *Salmonella* spp. and *Listeria monocytogenes* revealed that *Lactobacillus salivarius* (LAB 59) was the LAB isolate that exhibited the best inhibitory activity against both pathogens.

Antagonistic activity on chicken skin. Based on the results from the broth experiments, *Lactobacillus salivarius* (LAB 59) was chosen to test its antagonistic action against *Salmonella* spp. and *Listeria monocytogenes* on chicken skin. Its population remained practically unchanged over the 6 days storage, as with the broth experiment. On the contrary, the pathogens were affected by the presence of *Lactobacillus salivarius* and on the 6th day there was a statistically significant reduction ($P \leq 0.05$) of 0.54 log CFU/cm^2 for *Salmonella spp.* and 0.71 log CFU/cm^2 for *Listeria monocytogenes* populations compared to the samples that were inoculated only with the pathogens (Table 2). It is worth mentioning that the UV treatment of chicken skin was found to be effective as a means of reducing the microbial counts since *Salmonella* spp., *Listeria monocytogene,* and LAB were not detected. However, *Salmonella* spp. and *Listeria monocytogenes* were isolated from the chicken skin that was not UV treated at a population of 10^3 CFU/cm^2, whereas LAB were isolated at a population of 10^5 CFU/cm^2.

Antagonistic activity on chicken meat. The results were similar to those obtained in the previous experiment on chicken skin, and on the 6th day there was a significant reduction ($P \leq 0.05$) of 0.51 log CFU/cm^2 for *Salmonella* spp. and 0.67 CFU/cm^2 for *Listeria monocytogenes* populations when inoculated together with *Lactobacillus salivarius*, compared to the samples that were inoculated only with the pathogens (Table 3). *Salmonella* spp., *Listeria monocytogenes*, and LAB were not detected on the chicken meat samples that were kept intact.

The sensory evaluation of chicken meat revealed that the high cells counts of the LAB inocula had no negative effect on the odour and slime appearance.

DISCUSSION

The results demonstrate the potential of 7 strains of lactic acid bacteria, and especially of *Lactobacillus salivarius,* to be used as protective cultures against *Salmonella* spp. and *Listeria monocytogenes* on chicken. Broth experiments along with those with chicken skin and meat were carried out and revealed differences in the effectiveness of the LAB strains against pathogens. A more pronounced effect was observed during the broth experiments. On the 5th day of the experiment, the growth reduction of 1.12 log CFU/ml for *Salmonella* spp. and 1.48 log CFU/ml for *Listeria monocytogenes* was attributed to the presence of the most effective LAB strain, *Lactobacillus salivarius*. On chicken skin, the growth reduction on the 6th day caused by the same LAB strain was lower and did not exceed, the 0.54 log CFU/cm^2 for *Salmonella* spp. and 0.71 CFU/cm^2 for *Listeria monocytogenes*. The reduction on chicken meat was slightly lower for both pathogens, while the differences between chicken skin

and meat were not found statistically significant ($P > 0.05$). The properties of a broth medium can favour the antagonistic activity of the LAB strains and may explain their strong reducing effect on pathogens. This comes in accordance with the findings of Jones *et al*. (2008) who suggested that LAB behaviour in laboratory media may not necessarily be reproducible in the foods. Another explanation could be the higher inoculum level of both LAB and pathogens in broths compared to the chicken skin and meat.

The effectiveness of *Lactobacillus salivarius* against *Salmonella* spp. has been observed by several researchers (Garriga *et al*. 1998; Pascual *et al*. 1999; Zhang *et al*. 2007a,b). In these studies, *Lactobacillus salivarius* was isolated from the crop and/or the ceca of chickens and afterwards was given to chickens as a bacterial culture with the feed. The antibacterial effect of *L. salivarius* was attributed to the production of lactic acid together with other acidic compounds like organic acids (Garriga *et al*. 1998). Other researchers (Audisio & Apella 2006) found that *L. salivarius*, isolated from the crop of chickens, had a bactericidal effect on both *Salmonella* spp. and *Listeria monocytogenes*. The compound produced by *L. salivarius* which had this effect was showed to be different from organic acids and hydrogen peroxide and was considered to be a bacteriocin or a bacteriocin like substance.

In a similar study by Maragkoudakis *et al*. (2009), stock cultures of LAB were applied as protective cultures to chicken meat. The growth reduction observed on day 7 was 1.2 log CFU/g for *Salmonella* spp. and 0.7 log CFU/g for *Listeria monocytogenes*. These values were higher than those in our study and this can be explained by several factors. Different strains of LAB were used for *Salmonella* spp. (*L. fermentum*) and *Listeria monocytogenes* (*E. faecium*), the initial inocula levels of LAB were higher (10^7 CFU/g), and the experiment took place in ground chicken meat.

In our study, no significant differences were found in the population levels of LAB strains during all three experiments. Their storage at 7°C for 5 or 6 days did not affect their populations and revealed that LAB reproduction was not necessary for the inhibition of pathogens. These findings come in accordance with the observations of Amezquita and Brashears (2002) and Ruby and Ingham (2009) who suggested that the inhibition can occur in the absence of LAB growth because of the continuous production of inhibitory metabolites by the LAB during storage.

The sensorial evaluation at the end of the storage showed that the inoculation of chicken meat with LAB did not affect its sensory properties. This may be attributed to the fact that the number of LAB remained practically stationary throughout the 6 day storage. Actually, the surface of the chicken meat that was inoculated with the strain of *Lactobacillus salivarius* was clear and with a pleasant odour, whereas on the surface of chicken meat that was kept intact a slight slime appearance had started to grow. Our observations seem to be in accordance with the findings of Brashears *et al*. (1998) where the inoculation of chicken meat with *L. lactis* exerted control on the growth of psychrotrophic spoilage organisms. Poultry meat usually spoils due to the growth of these psychrotrophic spoilage organisms (Barnes 1976). Therefore, the application of *L. salivarius* to raw chicken meat may provide a useful means for inhibiting these organisms and extending the shelf life of the product.

The antagonistic activity of LAB isolated from chicken carcasses and mainly of *L. salivarius* against *Salmonella* spp. and *Listeria monocytogenes* was evaluated during this study. The presence of *L. salivarius* was found to affect significantly the growth of pathogens in broths, on chicken skin and meat. Its presence may not eliminate the population of pathogens, but along with other microbial hurdles and safety factors such as good hygiene practices may improve the microbiological safety of chicken products. Moreover, the sensorial evaluation proved that the addition of *L. salivarius* not only reduced the growth of pathogens but also inhibited the growth of slime on chicken meat and improved its overall appearance. The addition of substances to the chill tank of poultry in order to inhibit pathogens has been evaluated in several studies (Brashears *et al*. 1998). Our suggestion would be the addition of *L. salivarius* as a concentrated culture to the chill tank, among other microbial hurdles that can be applied, in order to improve the safety and to extend the shelf life of chicken products.

References

Amezquita A., Brashears M.M. (2002): Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. Journal of Food Protection, **65**: 316–325.

Audisio M.C., Apella M.C. (2006): Bacteriocin-like substance produced by *Lactobacillus salivarius* subsp. *salivarius* CRL1384 with anti-*Listeria* and anti-*Salmonella* effects. Research Journal of Microbiology, **1**: 61–69.

Barnes E.M. (1976): Microbiological problems of poultry at refrigeration temperatures. A review. Journal of the Science of Food and Agriculture, **27**: 777–782.

Brashears M.M., Reilly S.S., Gilliland S.E. (1998): Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. Journal of Food Protection, **61**: 166–170.

Brashears M.M., Amezquita A., Jaroni D. (2005): Lactic acid bacteria and their use in animal feeding to improve food safety. Advances in Food and Nutrition Research, **50**: 1–31.

EFSA (2010): The community summary report on trends and sources of zoonoses and zoonotic agents and foodborne outbreaks in European Union in 2008. EFSA Journal, **8**(1): 1496: 3–313.

Garriga M., Pascual M., Monfort J.M., Hugas M. (1998): Selection of lactobacilli for chicken probiotic adjuncts. Journal of Applied Microbiology, **84**: 125–132.

Holzapfel W.H., Geisen R., Schillinger U. (1995): Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. International Journal of Food Microbiology, **24**: 343–362.

Jay J.M. (1996): Microorganisms in fresh ground meats: The relative safety of products with low versus high numbers. Meat Science, **43**: 59–66.

Jones R.J., Hussein H.M., Zagorec M., Brightwell G., Tagg J.R. (2008): Isolation of lactic acid bacteria with inhibitory against pathogens and spoilage organisms associated with fresh meat. Food Microbiology, **25**: 228–234.

Kostrzynska M., Bachand A. (2006): Use of microbial antagonism to reduce pathogen levels on produce and meat products: a review. Canadian Journal of Microbiology, **52**: 1017–1026.

Laursen B.G., Bay L., Cleenwerck I., Vancanneyt M., Swings J., Dalgaard P., Leisner J.J. (2005): *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: phenotypic and genotypic characterization. Systematic and Applied Microbiology, **28**: 151–164.

Leroy F., Verluyten J., De Vuyst L. (2006): Functional meat starter cultures for improved sausage fermentation. International Journal of Food Microbiology, **106**: 270–285.

Loretz M., Stephan R., Zweifel C. (2010): Antimicrobial activity of decontamination treatments for poultry carcasses: A literature survey. Food Control, **21**: 791–804.

Lucke F.K. (2000): Utilization of microbes to process and preserve meat. Meat Science, **56**: 105–115.

Maragkoudakis P.E., Mountzouris K.C., Psyrras D., Cremonese S., Fischer J., Cantor M.D., Tsakalidou E. (2009): Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. International Journal of Food Microbiology, **130**: 219–226.

Muthukumarasamy P., Han J., Holley R. (2003): Bactericidal effects of *Lactobacillus reuteri* and allyl isothiocyanate on *Escherichia coli* O157:H7 in refrigerated ground beef. Journal of Food Protection, **66**: 2038–2044.

Pascual M., Hugas M., Badiola J.I., Monfort J.M., Garriga M. (1999): *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Applied and Environmental Microbiology, **65**: 4981–4986.

Ruby J.R., Ingham S.C. (2009): Evaluation of potential for inhibition of growth of *Escherichia coli* O157:H7 and multidrug-resistant *Salmonella* serovars in raw beef by addition of a presumptive *Lactobacillus sakei* ground beef isolate. Journal of Food Protection, **72**: 251–259.

Sakaridis I., Soultos N., Iossifidou E., Koidis P., Ambrosiadis I. (2011a): Prevalence and antimicrobial resistance of *Salmonella* serovars from chicken carcasses in Northern Greece. Journal of Food Safety, **31**: 203–210.

Sakaridis I., Soultos N., Iossifidou E., Papa A., Ambrosiadis I., Koidis P. (2011b): Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated from chicken carcasses. Journal of Food Protection, **74**: 1017–1021.

Sakaridis I., Soultos N., Dovas C.I., Papavergou E., Ambrosiadis I., Koidis P. (2012): Isolation and identification of lactic acid bacteria from chicken carcasses with inhibitory activity against *Salmonella* spp. and *Listeria monocytogenes*. Anaerobe, **18**: 62–66.

Senne M.M., Gilliland S.E. (2003): Antagonism action of cells of *Lactobacillus delbrueckii* subsp. *lactis* against pathogenic and spoilage microorganisms in fresh meat systems. Journal of Food Protection, **66**: 418–425.

Vermeiren L., Devlieghere F., Debevere J. (2004): Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. International Journal of Food Microbiology, **96**: 149–164.

Zhang G., Ma L., Doyle M.P. (2007a): Potential competitive exclusion bacteria from poultry inhibitory to *Campylobacter jejuni* and *Salmonella*. Journal of Food Protection, **70**: 867–873.

Zhang G., Ma L., Doyle M.P. (2007b): Salmonellae reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristatus*. Journal of Food Protection, **70**: 874–878.

Zolman J. (1993): Biostatistics: Experimental Design and Statistical Inference. Oxford University Press, Inc., New York.

Received for publication October 23, 2012
Accepted after corrections May 24, 2013

Corresponding author:

Dr Ioannis Sakaridis, Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, Department of Hygiene and Technology of Foods of Animal Origin, 54 124 Thessaloniki, Greece; E-mail: ioannis_s_@hotmail.com; jsakarid@vet.auth.gr