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Behaviour of *Listeria monocytogenes* LMG 10470 in Poultry Meat and its Control by the Bacteriocin Plantaricin UG 1

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Abstract: Growth of the *Listeria monocytogenes* LMG10470 (*L. monocytogenes*) pathogen were prevented by partially purified plantaricin UG1 within almost 2-3 days in uncooked and cooked poultry meat and dry mortadella samples of chicken and turkey. Inoculation of the experimental samples of cooked and uncooked meat and dry mortadella of chicken and turkey by the *Lactobacillus plantarum* UG1 L*act. plantarum* UG1) cells producing the bacteriocin plantaricin UG1 resulted in inactivation of the all experimental *L. monocytogenes* cells within almost 3-4 days. By further storage of the treated samples, a slight resurgence of *L. monocytogenes* growth was observed in almost all poultry samples treated with plantaricin UG1, but was not observed in all poultry samples of cooked and uncooked meat and mortadella samples of chicken and turkey inoculated with the *Lact. plantarum* UG1 cells producing plantaricin UG1

Key words: *Listeria monocytogenes (L. monocytogenes), Lactobacillus plantarum* UG1 *(Lact. plantrumUG1)*, Bacteriocin, Plantaricin UG1, Poultry, Poultry Mortadella

Introduction

L. monocytogenes is present in soil, water, vegetables and intestinal contents of variety of birds, fish, insects and other animals (Mahmoued *et al*., 2003). *L. monocytogenes* is a food borne pathogen which is of major concern due to its ability to grow in a variety of foods at refrigeration temperatures (Farber and Peterkin, 1991). The poultry meat products have frequently been recognized to be contaminated with *L. monocytogenes* and were, therefore, considered as an important vehicle of human listerial infection (Mahmoued *et al*., 2003). Human listeriosis is a sporadic disease and many range from mild flu-like sickness to severe manifestations like meningoenceohalities. Groups of highest risk are pregnant women, neonates and peoples with immune compromised conditions (Demetrios and Antonios, 1996). The frequent association of *L. monocytogenes* with poultry (Schlyter *et al*., 1993; Enan, 2006 a,b) emphasize the importance for continued research to develop new improved strategies to control this pathogen in poultry as well as other foods. A potential means of preserving meat products against *L. monocytogenes* is the use of becteriocins produced by pediococci (Schlyter *et al*., 1993), carnobacteria (Mathieu *et al*., 1994) and lactococci (Nettles and Barefoot, 1993). However, nothing is known in the literature about inhibition of *L. monocytogenes* in meat products of beef and poultry by *lactobacillus plantarum* bacteriocin. In an attempt to fill this gap, the bacteriocin plantaricin UG1 produced by *Lact. plantarum* UG1 inhibited *L. monocytogenes in vitro* and in beef meat (Enan, 2000; Enan *et al*., 2002) and the present work was undertaken to study the growth of *L. monocytogenes* LMG 10470 in cooked and uncooked meat and mortadella samples of chicken and turkey and its control by the bacteriocin plantaricin UG1.

Materials and Methods

Cultures and media: *Lact. plantarum* UG1, the producer of the bacteriocin plantaricin UG1 has been isolated and characterized as described previously (Enan *et al*., 1994 a,b). *Lact. plantarum* UG1 was grown in De Man, Rogosa and Sharpe medium (MRS broth) (De Man *et al*., 1960) and was subcultured every one week in MRS broth *L. monocytogenes* LMG 10470 was kindly provided from Department of Food Technology and Food Preservation, Faculty of Agriculture and Applied Biological Sciences, University of Gent, Belgium. It was maintained as frozen stocks in trypticase soy broth (Difco) plus 10% glycerol and was subcultured every two weeks in trypticase soy broth (Joerger and Klaenhammer, 1986). Growth of *L. monocytogenes* was measured onto trypticase soy broth (Oxoid) as cfu/g. Growth values were taken from three replicates.

Food samples: Fresh meat slices of chicken and turkey were prepared. They were minced by their grinding in sterile mincer. Half of minced amounts were cooked in steam oven. 100 g portions of cooked samples and 100 g portions of uncooked samples were placed aseptically

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in sterile plastic bags and were used in the experiments (Enan, 2006 b,c).

Preparation of partially purified plantericin UG1: *Lact. plantarum* UG1, the producer of plantaricin UG1, was grown in MRS broth for 16h at 30°C. Cell free supernatants were obtained by centrifuging the culture (10000xg for 10 min. at 4°C). The pH value of that cell free supernatants was then adjusted to pH 6.5. This is to exclude the inhibitory activity due to organic acids. This pH adjusted cell free supernatant was subjected to ammonium sulfate precipitation (50%) as described previously (Enan, 2000). The ammonium sulfate precipitates (surface pellicels and pellets) were recovered in 10mM potassium phosphate buffer, pH 6.5, and dialyzed against the same buffer for 24h at 4°C in visking dialysis membrane (Enan *et al.*, 1996). This partially purified plantaricin UG1 was sterilized by filtration through cellulose membrane filters (0.45µm; Milipore, Amicon) and was used in the experiments. The activity of this partially purified plantaricin UG1 was measured as described previously (Biswas *et al.*, 1991). About 8200 AU/ml of this partially purified plantaricin UG1 were showed to saturate the absorptions sites of $2x10^7$ cfu/ml of the experimental *L. monocytogenes* strain. This bacteriocin concentration was, therefore, used in the present experiments.

Inhibition of *L. monocytogenes* by the bacteriocin producing strain, *Lact. plantarum*, in food samples: Sterilized plastic bags, each containing 100 g food samples (cooked meat, uncooked meat and mortadella samples of chicken and turkey) were inoculated with about $2x10^7$ cfu/g of *L. monocytogenes* and about $2x10^7$ cfu/ml of the plantaricin and about $2x10^7$ cfu/ml of the plantaricin UG1 producing strain, lact plantarum UG1. Control samples were inoculated by the *L. monocytogenes* cells, but not by *Lact. plantarum* UG1. Samples and controls were shaken vigorously by hand to distribute the inocula and were then stored at 15 °C for two weeks. At appropriate time intervals, 5 g samples were removed, serially diluted and the *L. monocytogenes* viable cells were determined as cfu/g onto trypitcase soy agar medium (Enan *et al.*, 2002; Enan And Al-Amry., 2006).

Inhibition of *L. monocytogenes* in food samples by partially purified plantaricin UG1: Sterilized plastic bags, each containing 100 g aliquot of either cooked meat or uncooked meat and/or dry mortadella of chicken and turkey were inoculated by cell suspension of the *L. monocytogenes* strain to give 2×10^7 cfu/g final concentration and then shaken vigorously by hand to distribute the inocula. The above inoculated samples were treated with 8200 AU/ml partially purified plantaticin UG1 and were shaken again to mix the bacteriocin with the inoculated samples. Control samples were inoculated by the *L. monocytogenes* cells, but not treated with the bacteriocin plantaricin UG1. Samples and controls were incubated at 15 °C for two weeks. After appropriate time intervals, 5 g aliquots were taken from either samples or controls and analyzed for viable counts of *L. monocytogenes* as described previously (Enan *et al.*, 2002; Enan *et al.*, 2006, b, c).

Results

Table 1 shows the growth values of the experimental *L. monocytogenes* pathogen in uncooked meat samples of poultry (chicken and turkey) treated with the *Lact. plantarum* UG1 cells and in controls. The initial cell population of *L. monocytogenes* (2×10^7 cfu/g) were being declined completely within almost 3 days in the treated samples, but were increased to almost 4.2-8×10^9 cfu/g in controls. As given in Table 2, the cooked meat samples of chicken and turkey treated with the *Lact. plantarum* UG1 cells and their controls gave almost similar growth values of the *L. monocytogenes* cells to that obtained above in the uncooked samples. No. regrowth of the *L. monocytogenes* cells was obtained in either uncooked or cooked samples of poultry meat (chicken and turkey) treated with the *Lact. plantarum* UG1 cells producing the bacteriocin plantaricin UG1 throughout 2 weeks of storage at 15 °C. The inhibition of *L. monocytogenes* by the plantaricin UG1 producer, *Lact. plantarum* UG1, in dry mortadella samples of ckicken and turkey is shown in Table 3. The *Lact. plantarum* UG1 cells prevented growth of *L. monocytogenes* in mortaldella samples of chicken and turkey, whereas in controls, the initial cell population of the *L. monocytogenes* cells (2×10^7 cfu/g) were increased to almost 2.1 – 2.8 $\times$ 10^8 cfu/g). No regrowth of the *L. monocytogenes* cells was observed in the treated samples throughout 2 weeks of storage.

The inhibition of *L. monocytogenes* by partially purified plantaricin UG1 in uncooked; cooked meat samples of chicken and turkey was showed in Table 4, Table 5 respectively. In controls, viable cell population of the *L. monocytogenes* cells were being increased from $2x10^7$ cfu/g to $4.2x10^9$ cfu/g; $8x10^9$ cfu/g in uncooked meat of chicken; turkey respectively (Table 4) and to $7.7x10^9$ cfu/g; $6.7x10^9$ cfu/g in cooked meat of chicken; turkey respectively (Table 5). However, in samples treated with partially purified plantaricin UG1, viable cells of the *L. monocytogenes* declined sharply and no growth of the *L. monocytogenes* cells were observed within 36 – 48 h in both uncooked and cooked poultry (chicken and turkey) (Table 4, 5). A slight regrowth of *L. monocytogenes* was observed after one week of storage.

Table 6 shows the effect of partially purified plantaricin UG1 on growth of the *L. monocytogenes* pathogen in mortadella samples of chicken and turkey. Viable cell population of *L. monocytogenes* were increased from

Table 1: Growth of the *L. monocytogenes* LMG 10470 strain in uncooked meat of chicken and turkey and its control by the plantaricin UG1 strain: Lactobacillus plantarum UG1
Colony forming units (cfu/g) of *L. monocytogenes* in the treated uncooked meat samples (chicken and turkey) and controls (uncooked meat without bacteriocin)

Time (h)	Uncooked meat of chicken		Uncooked meat of turkey	
	Control	Sample	Control	Sample
Zero	2×10^7	2×10^7	2×10^7	2×10^7
12	4.2×10^7	3×10^6	5.1×10^7	3.3×10^5
24	7.3×10^7	1.1×10^4	8.2×10^7	6×10^4
36	9.8×10^7	3×10^3	2.8×10^8	3.2×10^3
48	2.1×10^8	3.8×10^2	8.1×10^8	2×10^2
60	5.2×10^8	1.2×10^2	2.1×10^9	10
72	8×10^8	1×10^2	6.2×10^9	Zero
84	4.1×10^9	zero	7.1×10^9	zero
96	4.2×10^9	zero	8×10^9	zero

Table 2: Growth of the L. moncytogenes LMG 10470 strain in cooked meat of chicken and turkey and its control by the plantaricin UG1 producing strain: Lactobacillus plantarum UG1.
Colony forming units (cfu/g) of *L. monocytogenes* in the treated cooked meat samples (chicken and turkey) and controls (cooked meat without bacteriocin)

Time (h)	Cooked meat of chicken		Cooked meat of turkey	
	Control	Sample	Control	Sample
Zero	2×10^7	2×10^7	2×10^7	2×10^7
12	5.1×10^7	6×10^5	6.1×10^7	3.6×10^4
24	8.2×10^7	3×10^3	9.6×10^7	2×10^2
36	3.2×10^8	1.1×10^3	3.7×10^8	1×10^2
48	7.1×10^8	2.2×10^2	6.8×10^8	10
60	1×10^9	1×10^2	9.2×10^8	10
72	4.4×10^9	10	2.6×10^9	Zero
84	7.8×10^9	zero	6.6×10^9	zero
96	7.7×10^9	zero	6.7×10^9	zero

Table 3: Growth of the L. moncytogenes LMG 10470 strain in dry mortadella samples of chicken and turkey and its control by the plantaricin UG1 producing strain: Lactobacillus plantarum UG1.
Colony forming units (cfu/g) of *L. monocytogenes* in the bacteriocin treated samples of chicken and turkey and controls (samples without bacteriocin)

Time (h)	Chicken mortadella		Turkey mortadella	
	Control	Sample	Control	Sample
Zero	2×10^7	2×10^7	2×10^7	2×10^7
12	3.1×10^7	1.6×10^6	2.8×10^7	4.2×10^5
24	4.4×10^7	2.2×10^4	3.6×10^7	7.8×10^3
36	6.3×10^7	4.3×10^3	5.8×10^7	3.6×10^2
48	8.8×10^7	2.8×10^2	7.2×10^7	100
60	1×10^8	1.6×10^2	9.6×10^7	100
72	3.2×10^8	1.1×10^2	2×10^8	10
84	3×10^8	100	2×10^8	zero
96	2.8×10^8	zero	2.1×10^8	zero

2×10^7 cfu/g to almost 2.1-2.8 $\times 10^8$ cfu/g in controls, but were decreased from 2×10^7 cfu/g to 2 - 9.1×10^2 cfu/g in the bacteriocin treated samples of both chicken and turkey mortadella (Table 6).

Discussion

The *L. monocytogenes* pathogen is frequently found on raw and processed poultry and can grow on processed poultry products stored under refrigeration (Schlyter *et al*., 1993, Lunden *et al*., 2003). This emphasize the importance for continued research to develop improved strategies to control the *L. monocytogenes* pathogen in poultry and other foods. The traditional methods employed to control *L. monocytogenes* pathogen in poultry are using chemical additives which are human harmful (Schlyter *et al*., 1993; Berrang *et al*., 2005). This clearly indicates that there is a need to develop a safe biological methods for controlling the *L. monocytogenes* pathogen in poultry meat. In this regard, lactic acid bacteria bacteriocins, are natural antimicrobial agents in foods (Nettles and Barefoot, 1993; Mattila *et al*., 2003; Enan *et al*., 2006 a, b). The only bacteriocin produced by lactic acid bacteria and currently used in the food industry is nisin which has a limited applicability because of its instability at neutral pH (Varadaraj *et al*., 1993). For the above reasons, the bacteriocin plantaricin UG1 produced by *Lact. plantarum* UG1 have been used for controlling the *L. monocytogenes* pathogen, in this investigation, in mortadella and meat samples of uncooked and cooked chicken and turkey. This is to complete our previous work on the efficiency of the bacteriocin plantaricin UG1 in food biopreservation (Enan *et al*., 1994 a,b; Enan, 2000; Enan *et al*., 2002; Abdel-Salam *et al*., 2004; Enan *et al*., 2004; Enan and Saad., 2005; Enan and Al-Amry., 2006; Enan *et al*., 2006 a,b,c).

In the present study, partially purified plantaricin UG1 had a higher antilisterial activity than *Lact. plantarum* UG1 cells inoculated in poultry meat samples (chicken and turkey). This could be attributed to many factors in poultry meat which can affect bacteriocin production by *Lact. plantarum* UG1. These factors are assimilated in pH value and fibers of poultry meat (Rekhif *et al*., 1994). The inhibition of the *L. monocytogenes* cells by the *Lact. plantarum* UG1 cells noticed in this study was due to plantaricin UG1 but not to lactic acid produced by the *Lact. plantarum* UG1 culture as the growth value of the *L. monocytogenes* cells were unaffected by plantaricin UG1 negative variant (Enan *et al*., 2002). Since *Lact. plantarum* UG1 was isolated from dry sausage (Enan *et al*., 1996). It grew in poultry mortadella (in this study) and its antilisterial activity in this poultry mortadella will be quite promising for the development of a wider application of this bacteriocin producing strain (*Lact. plantarum* UG1) in poultry meat with control of this pathogen.

The results employed herein have indicated that poultry meat samples inoculated with *Lact. plantarum* UG1 didn't show resurgence of the *L. monocytogenes* growth by further storage of samples at 15°C. This could be due to presence of the *Lact. plantarum* UG1 metabolites

Table 4: Inhibition of L. monocytogenes LMG 10470 by partially purified plantaricin UG1 in uncooked poultry meat (chicken and turkey).
Colony forming units (cfu/g) of *L. monocytogenes* in the bacteriocin treated uncooked meat samples (chicken and turkey) and controls (samples without bacteriocin)

Time (h)	Uncooked meat of chicken		Uncooked meat of turkey	
	Control	Sample	Control	Sample
Zero	$2x10^7$	$2x10^7$	$2x10^7$	$2x10^7$
12	$4.2x10^7$	$2x10^4$	$5.1x10^7$	$3.1x10^4$
24	$7.3x10^7$	$3.6x10^2$	$8.2x10^7$	$2.2x10^2$
36	$9.8x10^7$	$1x10^2$	$2.8x10^8$	$1x10^2$
48	$2.1x10^8$	zero	$8.1x10^8$	100
60	$5.2x10^8$	zero	$2.1x10^9$	zero
72	$8x10^8$	zero	$6.2x10^9$	zero
84	$4.1x10^9$	zero	$7.1x10^9$	zero
96	$4.2x10^9$	10	$8x10^9$	10

Table 5: Inhibition of *L. monocytogenes* LMG 10470 by partially purified plantaricin UG1 in cooked poultry meat (chicken and turkey).
Colony forming units (cfu/g) of *L. monocytogenes* in the bacteriocin treated cooked meat samples (chicken and turkey) and controls (samples without bacteriocin)

Time (h)	Cooked meat of chicken		Cooked meat of turkey	
	Control	Sample	Control	Sample
Zero	$2x10^7$	$2x10^7$	$2x10^7$	$2x10^7$
12	$5.1x10^7$	$3x10^3$	$6.1x10^7$	$2x10^4$
24	$8.2x10^7$	$2x10^2$	$9.6x10^7$	$3.1x10^3$
36	$3.2x10^8$	zero	$3.7x10^8$	$1.2x10^2$
48	$7.1x10^8$	zero	$6.8x10^8$	100
60	$1x10^9$	zero	$9.2x10^8$	zero
72	$4.4x10^9$	zero	$2.6x10^9$	zero
84	$7.8x10^9$	zero	$6.6x10^9$	zero
96	$7.7x10^9$	zero	$6.7x10^9$	10

Table 6: Inhibition of *L. monocytogenes* LMG 10470 by partially purified plantaricin UG1 in dry mortadella of chicken and turkey.
Colony forming units (cfu/g) of *L. monocytogenes* in the bacteriocin treated mortadella samples (chicken and turkey mortadella) and controls (samples without bacteriocin)

Time (h)	Chicken mortadella		Turkey mortadella	
	Control	Sample	Control	Sample
Zero	$2 x10^7$	$2 x10^7$	$2 x10^7$	$2 x10^7$
12	$3.1x10^7$	$1.1x10^6$	$2.8x10^7$	$1.3x10^6$
24	$4.4x10^7$	$3.2x10^5$	$3.6x10^7$	$2.6x10^5$
36	$6.3x10^7$	$6x10^4$	$5.8x10^7$	$7x10^4$
48	$8.8x10^7$	$3.2x10^3$	$7.2x10^7$	$6.8x10^3$
60	$1x10^8$	$9.8x10^2$	$9.6x10^7$	$3.8x10^3$
72	$3.2x10^8$	$2x10^2$	$2x10^8$	$9.1x10^2$
84	$3x10^8$	zero	$2x10^8$	$2x10^2$
96	$2.8x10^8$	zero	$2.1x10^8$	zero

thoroughly (bacteriocin, H_2O_2, organic acids) in the treated samples can inactivate the respective growing cells of *L. monocytogenes* (Nettles and Barefoot, 1993). This would appear that this offers a very promising means for the biological biocontrol of the *L. monocytogenes* cells without restoring to more severe physical treatments of the foods.

The addition of partially purified plantaricin UG1 to poultry meat and mortadella samples of chicken and turkey prevented the experimental *L. monocytogenes* cells throughout one week, but thereafter a slight regrowth of the pathogen was observed. This could be attributed to inactivation of plantaricin UG1 by extracellular molecules produced by the *L. monocytogenes* cells or due to adsorption of plantaricin UG1 on meat fibers (Vignolo *et al.*, 2000; Bouttefory and Milliere, 2000). Consequently the use of plantaricin UG1 mixed with nisin as an antilisterial mixture is recommended and will be promised in food biopreservation. This nisin-plantaricin UG1 mixture inhibited the regrowing variants of *L. monocytogenes in vitro* and in food without appearance of cross resistance between plantaricin UG1 and nisin (Enan, 2006 b). Also, the use of *Lact. plantarum* UG1 as a starter and protective culture may avoid the need for adding chemical inhibitors, a practice which has become interestingly unpopular as the problem of chemical food additives receives more scrutiny from food control agencies.

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