Antilisterial Activity of Plantaricin UG1 during Manufacture of Zabady and Kareesh Cheese: Two Arabian Dairy Products

Abdulluah A. Altalhi

Biology Department, Faculty of Science, Taif University, Box. 888, Taif, Saudi Arabia

ABSTRACT

Here, plantaricin UG1 was shown to inhibit Listeria monocytogenes LMG10470 (L. monocytogenes) cells. The inhibition was not caused by lactic acid produced by the bacteriocin producing strain Lactobacillus plantarum UG1 (Lact. plantarum). Partially purified plantaricin UG1 had a higher antilisterial activity than Lact. plantarum cells in both Zabady and Kareesh cheese samples during their maturation. Compared to the antilisterial activity in Kareesh cheese, plantaricin UG1 showed a faster effect during Zabady manufacture. (Int J Biomed Sci 2008; 4 (4): 319-322)

Keywords: Listeria monocytogenes LMG 10470 (L. monocytogenes); Lactobacillus plantarum UG1 (Lact. Plantarum); plantaricin UG1; bacteriocin; Zabady; Kareesh Cheese

INTRODUCTION

Listeria monocytogenes (L. monocytogenes) is a gram positive rod that is catalase positive and shows a characteristic tumbling mobility (1). It has long been recognized as a veterinary pathogen and in humans causes a disease known as listeriosis particularly in neonates or immunocompromised hosts (2, 3). A number of listerial outbreaks related to dairy products have been described (4). Two sporadic cases of listerial meningitis were also attributed to consumption of dairy products (5). L. monocytogenes is able to grow in dairy products at various acidic conditions (PH3.5-7) and at various incubations temperatures (10°C-45°C) (6). Hence, inhibition of L. monocytogenes by a safe lactic acid bacterium or its metabolites is of interest. In this regard the bacteriocin planetaricin UG1 produced by Lact. plantarum inhibited some food–borne pathogens including L. monocytogenes in vitro (3, 6, 7, 8, 9). The bacteriocin plantaricin UG1 was active against L. monocytogenes at acidic and neutral PH values (pH3.5-7) and over a wide temperature range (Zero-90°C) (10, 11, 12). The present work was undertaken to inhibit growth of L. monocytogenes during maturation of two Arabian dairy products (Zabady and kareesh cheese) by the bacteriocin plantaricin UG1.

MATERIALS AND METHODS

Cultures and media: L. monocytogenes LMG10470 was provided from LMG culture collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium. It was counted on trypticase soy broth (13). Lact. plantarum UG1, the producer of plantaricin UG1 was provided kindly from prof. Dr. Gamal Enan at Botany Department, Faculty of Science, Zagazig University, Egypt. The plantaricin UG1 negative variant (BAC) was obtained as described previously (3, 8). Lact. plantarum UG1 and its BAC were grown in De Man, Rogosa and Sharpe medium (MRS) (14). Competitive growth of L. monocytogenes and Lact. plantarum UG1 cells (wild strain and BAC variant) were tested in BHI broth (Oxoid). Competitive growth of the zabady and Kareesh cheese starter cultures were also tested in BHI broth. Growth values were tested on agar
plates (tryplic soy agar for listerias cells and MRS agar for Lact. plantarum and its BAC strain). The results were obtained from three replicates.

Preparation of partially purified plantaricin UG1: Lact plantarum was grown in MRS broth for 16 h at 30°C. Cell-free supernatant was abtainned by centrifuging the culture (10000 xg. for 10 min at 4°C). The pH value of cell free supernatant was then adjusted to 6.5. This was to exclude the inhibitory activity due to organic acids. This pH-adjusted cell free supernatants were subjected to ammonium sulphate precipitation (50% saturation) as described previously (7). The ammonium sulphate precipitates (surface pellicels and pellets) were recovered in 10 mM potassium phosphate buffer, pH6.5, and dialyzed against the same buffer for 24 h at 4°C (13). The partially purified plantaricin UG1 was sterilized by filtration through cellulose membrane filters (0.45 µm, Milipore, Amicon). The activity of partially purified plantaricin UG1 was measured as described previously. One of this partially purified plantaricin UG1 appeared to contain 22880 AU/ml.

Inhibition of L. monocytogenes by bacteriocin producing Lact. plantarum and partially purified plantaricin UG1 during Zabady maturation

Zabady is an Arabian Yogurt. It was manufactured as follows: Mixture of fresh buffalo’s and cow’s milk (1:1) was heated at 80-82°C for 20 min. and cooled to 40°C. The milk was inoculated with 2% v/v of Zabady starter culture (mixture of Lactobacillus bulgaricus and Streptococcus cremories, Mircen Culture Collection, Ain Shams University, Egypt), mixed well, distributed to 150 ml scrow cremories (mixture of Lact plantarum and Streptococcus cremories), and were classifed three series. First; second series were inoculated with 2 × 10^6 cfu/ml of L. monocytogenes and were classifed into three series. First; second series were inoculated with about 2 × 10^6 cfu/ml of L. monocytogenes (the bacteriocin producer strain) and BAC, respectively. Third series of bottles were treated with about 22880 AU/ml of partially purified plantaricin UG1 (6). The contents of bottles were mixed well by hand. Bottles were then inoculated with 2 × 10^6 cfu/ml of L. monocytogenes during three days until curdled well. 5gm portions were taken at regular time intervals and analysed for viable cunts (cfu/ml) of L. monocytogenes as described above.

Inhibition of L. monocytogenes by bacteriocin producing Lact. plantarum and partially purified plantaricin UG1 during during kareesh cheese manufacture

Kareesh cheese is an Arabian dairy product. It was made as follows: Fresh defatted cow’s milk was heated at 85°C for 10 min. and then cooled to room temperature (approximately 25°C). The sterile milk was inculcated with 5% v/v mixed starter culture of Lactobacillus bulgaricus and Streptococcus cremories, Mircen Culture Collection, Ain Shams University, Egypt. It was also inculcated with about 2 × 10^9 cfu/ml of L. monocytogenes, mixed well and distributed to 2 L scrow capped plastic containers. Those 2 L samples were inculcated with about 2 × 10^6 cfu/ml of either Lact. plantarum (the wild strain) or BAC. Other series of 2 L samples were treated with about 22880 AU/ml of partially purified plantaricin UG1 (8). Samples were inculcated at 37°C until coagulation (2 days). The curd was scooped into mould(s) and was left to drain for 8 days. 5 g portions were removed every day throughout 10 days of maturation (before and after curd formation). Serial dilutions were prepared and viable cunts (cfu/ml) of L. monocytogenes were determined.

### RESULTS

In a preliminary experiment, growth of L. monocytogenes in BHI broth inculcated with either Zabady starter or Kareesh cheese starter cultures were tested. The results showed nearly comparable growth values of listerias cells in the absence of starter cultures. Growth of L. monocytogenes in Zabady samples (during maturation) inculcated by either Lact. plantarum or BAC strain were tested. Results are given in Table 1. L. monocytogenes alone (control) was increased from 2 × 10^6 cfu/ml to 9.7 × 10^9 cfu/ml within 4 days, whereas listerias growth was inhibited in presence of Lact. plantarum (the bacteriocin producer strain) reaching 7.7 × 10^9 after 72 h and 1.1 × 10^6 after 96

| Time (h) | Control without Lact. plantarum, UG1 | In the presence of Lact. Plantarum | BAC |
|----------|-------------------------------------|-----------------------------------|-----|
| 6        | 8 × 10^6                           | 6.2 × 10^6                        | 7.6 × 10^6 |
| 12       | 2 × 10^7                           | 1.1 × 10^6                        | 1.1 × 10^7 |
| 24       | 3.1 × 10^4                         | 1 × 10^6                          | 8.8 × 10^7 |
| 36       | 8.7 × 10^4                         | 2.6 × 10^5                        | 2.1 × 10^8 |
| 48       | 1.1 × 10^9                         | 1.1 × 10^5                        | 9.2 × 10^6 |
| 60       | 5.8 × 10^9                         | 8 × 10^4                          | 1 × 10^9  |
| 72       | 6.8 × 10^9                         | 7.7 × 10^3                        | 3.1 × 10^9 |
| 84       | 9 × 10^9                           | 3 × 10^3                          | 5 × 10^9  |
| 96       | 9.7 × 10^9                         | 1.1 × 10^2                        | 8.1 × 10^9 |
The BAC strain failed to inhibit *L. monocytogenes* and growth values of listerias cells were comparable to *Lact. plantarum*. Addition of partially purified plantaricin UG1 to the raw milk at the beginning of Zabady manufacture resulted in a marked bactericidal effect with reduction in viable counts of *L. monocytogenes* by four log cycles after 72 h (Table 2). No growth of listerias cells was obtained after 84 h (Table 2). However, the difference in colony counts between the treated samples and controls increased to reach $9 \times 10^9$ cfu/ml after 84h (Table 2).

As given in Table 3, inoculation of the raw milk used for Kareesh cheese at the beginning of manufacture processes resulted in a gradual decrease of the viable cell population of *L. monocytogenes* reaching $2.3 \times 10^7$ cfu/ml after 4 days and $9.3 \times 10^6$ cfu/ml after 6 days and $2 \times 10^5$ cfu/ml after 10 days whereas growth of *L. monocytogenes* alone (control) was increased from $2 \times 10^6$ cfu/ml to $2 \times 10^9$ cfu/ml within 10 days. A significant bactericidal effect of partially purified plantaricin UG1 was obtained during maturation of Karesh cheesse samples (Table 4). No growth of listerias cells was observed in Kareesh cheese after 7 days of storage. However, growth of *L. monocytogenes* cells in controls was increased from $2 \times 10^6$ cfu/ml to $2 \times 10^9$ cfu/ml within 10 days.

**DISCUSSION**

Bacteriocins are a group of antimicrobial proteins produced by Gram-positive bacteria (15). Use of lactic acid bacteria bacteriocins has been suggested a way to prevent spoilage in dairy products (16). The only bacteriocin produced by lactic acid bacteria and currently used in dairy industry is nisin which is produced by *Lactococcus Lactis ssp lactis* and has a limited applicability because of its instability at neutral pH (17). In an attempt to fill this gap, our results showed that plantaricin UG1 produced by *Lact. plantarum* has a vigorous antilisterial activity in Zabady and Karesh cheese samples during their maturation.

**Table 2. Growth of *L. monocytogenes* during maturation of an Arabian Zabady samples treated with the bacteriocin plantaricin UG1**

| Time (h) | Zabady samples without plantaricin UG1 (control) | Zabady samples inoculated with plantaricin UG1 |
|---------|------------------------------------------------|---------------------------------------------|
| 6       | $8 \times 10^6$                                | $1.1 \times 10^5$                           |
| 12      | $2 \times 10^7$                                | $8.6 \times 10^4$                           |
| 24      | $3.1 \times 10^4$                              | $3.3 \times 10^4$                           |
| 36      | $8.6 \times 10^4$                              | $3.1 \times 10^4$                           |
| 48      | $2 \times 10^9$                                | $3 \times 10^3$                            |
| 60      | $5.7 \times 10^9$                              | $2.1 \times 10^2$                           |
| 72      | $6.8 \times 10^9$                              | $1.1 \times 10^2$                           |
| 84      | $9 \times 10^9$                                | Zero                                        |
| 96      | $9.3 \times 10^9$                              | Zero                                        |

**Table 3. Growth of *L. monocytogenes* during maturation of samples of an Arabian Kareesh cheese inoculated with the bacteriocin producer strain *Lact. Plantarum* UG1**

| Time (day) | Samples of Kareesh cheese without *Lact. plantarum* (control) | Samples of Kareesh cheese Inoculated by *Lact. plantarum* UG1 | BAC |
|------------|---------------------------------------------------------------|---------------------------------------------------------------|-----|
| zero       | $2 \times 10^6$                                              | $2 \times 10^6$                                              |     |
| 1          | $7 \times 10^7$                                              | $3.2 \times 10^4$                                            | $7 \times 10^7$ |
| 2          | $3 \times 10^4$                                              | $1.1 \times 10^4$                                            | $2.9 \times 10^4$ |
| 3          | $1.1 \times 10^4$                                            | $6.6 \times 10^4$                                            | $1.0 \times 10^4$ |
| 4          | $1.3 \times 10^6$                                            | $2.3 \times 10^4$                                            | $1.3 \times 10^6$ |
| 5          | $1.1 \times 10^4$                                            | $1.1 \times 10^4$                                            | $1.1 \times 10^4$ |
| 6          | $2.2 \times 10^9$                                            | $9.3 \times 10^3$                                            | $2.2 \times 10^9$ |
| 7          | $3.3 \times 10^8$                                            | $6 \times 10^3$                                              | $1.0 \times 10^8$ |
| 8          | $2.2 \times 10^9$                                            | $6.2 \times 10^3$                                            | $9.9 \times 10^8$ |
| 9          | $1.2 \times 10^5$                                            | $3.3 \times 10^4$                                            | $8.6 \times 10^5$ |
| 10         | $2 \times 10^9$                                              | $2 \times 10^4$                                              | $8 \times 10^9$ |

**Table 4. Growth of *L. monocytogenes* during maturation of an Arabian Kareesh cheese samples treated with plantaricin UG1**

| Time (day) | Kareesh Cheese samples without plantaricin UG1 (control) | Kareesh Cheese samples treated with plantaricin UG1 |
|------------|----------------------------------------------------------|-----------------------------------------------------|
| zero       | $2 \times 10^6$                                          | $2 \times 10^6$                                     |
| 1          | $6.7 \times 10^7$                                         | $5 \times 10^6$                                     |
| 2          | $3.1 \times 10^4$                                         | $4 \times 10^4$                                     |
| 3          | $1.2 \times 10^5$                                         | $1.3 \times 10^5$                                   |
| 4          | $3 \times 10^9$                                           | $2 \times 10^2$                                     |
| 5          | $1.2 \times 10^9$                                         | $1 \times 10^2$                                     |
| 6          | $2.1 \times 10^9$                                         | $1 \times 10^2$                                     |
| 7          | $1.6 \times 10^9$                                         | Zero                                                |
| 8          | $2.2 \times 10^9$                                         | Zero                                                |
| 9          | $2.1 \times 10^9$                                         | Zero                                                |
| 10         | $2 \times 10^9$                                           | Zero                                                |
The experiments employed herein were designed to concur with the conditions that occasionally occur during maturation of either Zabady or Kareesh cheese which is often attained at room temperature in the Arabian countries. The plantaricin UGI concentration (22880 AU/ml) used in this study was chosen because it was appeared to be an ideal inhibitory one against *L. monocytogenes* (3).

The inhibition of listeria cells observed in this study was due to plantaricin UGI, and not to lactic acid produced by *Lact. plantarum* as the growth values of listerias were unaffected by the plantaricin UGI negative variant (*BAC*). This is consistent with many previous investigations (3, 8, 15, 18, 19). Partially purified plantaricin UGI had a higher antilisterial activity than *Lact. plantarum* UGI cells in both Zabady and Kareesh cheese at maturation. This could be due to adsorption of plantaricin UGI to both live and dead cells of its producer strain: *Lact. Plantarum* (3, 8, 9, 20).

Compared to the antilisterial activity in Kareesh cheese, plantaricin UGI showed a faster effect in Zabady samples during their maturation. This may be due to increased diffusion of this bacteriocin in Zabady as compared to the more solid curd of Kareesh cheese. These results are in agreement with previous published results (13).

The antilisterial activity of plantaricin UGI observed in this study during either Zabady or Kareesh cheese manufacture is quite promising for development of a wider application of the bacteriocin plantaricin UGI in the dairy industry. In addition, plantaricin UGI did not affect growth of starter lactic acid bacteria in either Zabady or Kareesh cheese during their maturation and hence this bacteriocin (plantaricin UGI) will be an ideal dairy preservative. This also could replace or reduce the use of dangerous chemical additives.

REFERENCES

1. Pucci MJ, Vedamuthu ER, Kunka BS and Vandenbergh PA. Inhibition of *L. monocytogenes* by bacteriocin PA.1 from *Pediococcus acidilactici* PAC.1. *Applied and Environ. Microbiol.* 1988; 54: 2349-2353.
2. Vignolo V, Balio J, Farias ME, Schilinger U, et al. Combined effect of bacteriocins on the survival of various in broth and meat system. *Current microbiol.* 2000; 41: 410-416.
3. Enan G. Nature and phenotypic characterization of plantaricin UGI resistance in *L. monocytogenes*. *J. Food Agricul. Environ.* 2006; 4: 105-108.
4. Bille J, Bannerman E, Jaquec C. *Listeria*, a new and promising one day system to identify *Listeria* isolates. *Appl. Environ. Microbiol.* 1992; 58: 1857-1860.
5. Azadian BS, Finner GT, Pearson AD. Cheese-born *Listeria meningitis* in immunocompetent patient. *Lancet.* 1989; 1: 322-323.
6. Enan G, Al-Hamidi AA, Abdel-Salam H. Influence of environmental conditions on plantaricin UGI production by *Lactobacillus plantarum* UGI in batch fermentation processes. *Alex. J. Pharmaceutical Science.* 2002; 17: 114-122.
7. Enan G, El-Essawy A, Uyttendaele M, Debevere J. Antibacterial activity of *L. plantarum* UGI: characterization, production and bactericidal action of plantaricin UGI. *Intern. J. Food Microbiol.* 1996; 30: 189-215.
8. Enan G. Control of the regrowing bacteriocin resistance variants of *L. monocytogenes* LMG 10470 *in vitro* and in food by nisin plantaricin UGI mixture. *Biotechnology.* 2006 b; 5: 143-147.
9. Enan G. Behaviour of *L. monocytogenes* LMG 10470 in poultry meat and its control by the bacteriocin plantaricin UGI. *Intern. J. Poultry Science.* 2006 c; 5: 335-339.
10. Enan G, EL. Sayed MA, Debevere J. Production and characterization of a bacteriocin by *Lactobacillus plantarum* UGI. Proceedings of the 8th Belgian Forum for Applied Biotechnology, Brugge, Belgium. 1994 a: 1795-1811.
11. Enan G, EL. Sayed MA, Debevere J. Influence of growth medium on *Lactobacillus plantarum* UGI growth and bacteriocin production. Proceedings of the 8th Belgian FORUM for Applied Biotechnology, Brugge, Belgium. 1994 a: 1795-1811.
12. Enan G, Abdel-Salam H, Al-Azouni E, Deevere J. Partial purification of plantaricin UGI: an antilisteriol bacitocin produced by *Lactobacillus plantarum* UGI. *New Egypt. J. Microbiol.* 2004; 9: 251-266.
13. Enan G. Inhibition of *Bacillus cereus* ATC 1459 by plantaricin UGI *in vitro* and in food. *Die Nahrung.* 2000; 44: 364-368.
14. De Man JC, Rogosa M, Shrepe ME. A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* 1960; 23: 130-138.
15. Nettles CG, Barefoot SF. Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. *J. Food Prot.* 1993: 45; 349-353.
16. Hoover DG. Bacteriocins with potential for use in foods. *New York: The bacteriocins, John Wiley and Stones.* 1994; 499-440.
17. Varadaraj MC, Devi N, Manjerck SP. Antimicrobial activity of neutralized culture filtrates of lactic acid bacteria from a cultured Indian milk product ‘Dahi’. *Int. J. Food Microbiol.* 1993; 20: 259-267.
18. Kalenhammer TR. Bacteriocins of lactic acid bacteria. *Biochemie.* 1988; 70: 337-349.
19. Laukova A, Szikkova S. Antagonistic effect of enterocin CCM 4231 from *Enterococcus faecium* on ‘bryndza’ a traditional Slovak dairy product from sheep milk. *Microbiological Research.* 2001; 1: 34-38.
20. Enan G, Al-Amri A. Novel plantaricin UGI production by *Lactobacillus plantarum* UGI in enriched whey permeate in batch fermentation processes. *J. Food Agriculture and Environment.* 2006; 4: 84-88.