

**Research Article**

## **PROTECTIVE EFFECT OF ENTEROCOCCAL BACTERIOCINS AGAINST *LISTERIA MONOCYTOGENES* IN CANTALOUPE AND WATERMELON JUICES**

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### **ABSTRACT**

Melons have been implicated in listeriosis outbreaks. In the present study, juices from cantaloupe and watermelon were inoculated with *Listeria monocytogenes* and then supplemented with three bacteriocin preparations, including enterocin EJ97 from *Enterococcus faecalis* and two bacteriocin preparations produced by *Enterococcus faecium* strains (SFB1 and SFB2). Viable *Listeria* counts during refrigeration storage for 7 days decreased below detectable levels for enterocin EJ97 and by a maximum 3.55 to 3.62 log cycles for SFB1 and SFB2 bacteriocin preparations, both in cantaloupe and in watermelon juice. The results highlight the potential of enterocins (and specially enterocin EJ97) as hurdles against *L. monocytogenes* in melon juices.

**Keywords:** *Bacteriocins, Listeria, Melon Juice*

### **INTRODUCTION**

The food borne pathogen *Listeria monocytogenes* is the causative agent of human listeriosis (Ghandi and Chikindas, 2007). In 2013, the EFSA reported 1,763 confirmed human cases of listeriosis, which represented an 8.6 % increase compared with 2012 (EFSA and ECDC, 2015). In the United States, *L. monocytogenes* is responsible for approximately 1,600 cases of food borne listeriosis annually, resulting in an estimated 1,500 hospitalizations and 260 deaths (Scallan *et al.*, 2011). Growth of *L. monocytogenes* has been demonstrated in a number of vegetable foods under refrigerated and ambient conditions (Harris *et al.*, 2003; Penteado and Leitao, 2004; Cobo-Molinos *et al.*, 2005, 2008). In 2011, a *Listeria monocytogenes* outbreak traced to whole cantaloupes caused 146 illnesses in 28 states and led to 32 deaths and 1 miscarriage, making it the deadliest U.S. outbreak of food borne illness to occur in the previous 25 years (CDC, 2012). Cantaloupe – indeed, all cut melon, such as watermelons and honeydews – are considered by the federal US government to be “potentially hazardous.” Since the melons grow on the ground, they are susceptible of becoming contaminated with bacteria in the soil, irrigation water, or in water runoff after a rain.

Bacteriocins are antimicrobial peptides of ribosomal synthesis produced by bacteria. Interest in bacteriocins as natural preservatives against food borne pathogens is not new, and a number of studies have been conducted to control *L. monocytogenes* and other food borne pathogens in food systems (Gálvez *et al.*, 2014). Previous studies from our research group allowed the isolation of enterococcal bacteriocins enterocin EJ97, and uncharacterized enterocins from seafood products (Gálvez *et al.*, 1998; Valenzuela *et al.*, 2010). The efficacy of bacteriocins greatly depends on the food borne pathogen and the food substrate, therefore bacteriocins need to be tested for each particular food system. The purpose of the present study was to test three enterococcal bacteriocins as hurdles against *L. monocytogenes* in cantaloupe and watermelon juices.

### **MATERIALS AND METHODS**

#### ***Bacterial Strains***

*Listeria monocytogenes* strain CECT4032 was from the Spanish Type Culture Collection (CECT, Burjasot, Valencia). The strain was propagated overnight in 1 ml brain-heart infusion broth (BHI, Scharlab, Spain). Cells from 1 ml overnight culture were collected by centrifugation (13.000 x g for 10

### **Research Article**

min), washed with sterile saline solution, and resuspended in 10 ml sterile saline solution before use for inoculation of rice milk.

Bacteriocin producing strains and *Enterococcus faecalis* S-47 strain used as control for bacteriocin assay were from our lab collection.

#### **Bacteriocin Preparation**

Three bacteriocin preparations (enterocin EJ97, and two bacteriocins from *E. faecium* strains (referred to as SFB1 and SFB2) isolated from seafood (Gálvez *et al.*, 1998; Valenzuela *et al.*, 2010) known to have strong anti-*Listeria* activity were used for assays.

For preparation of bacteriocin concentrates, strains were cultivated in phosphate-buffered BHI broth (1 liter each) overnight. After removal of cells by centrifugation (3,500 x g for 30 min), the bacteriocin from supernatants was recovered by ammonium sulfate precipitation at 70% saturation while being kept under refrigeration for 18 h.

Precipitates were collected by centrifugation (4,500 x g for 30 min) and resuspended in 20 ml phosphate buffer saline (PBS).

Bacteriocin concentrates were dialysed overnight using 2,000 molecular weight cut-off benzoylated dialysis tubing (SigmaAldrich, Madrid) and filtered through 0.22 µm pore size low protein binding filters (Millex GV; Millipore Corp., Belford, MA, USA) under sterile conditions.

Bacteriocin concentrates were serially diluted in sterile saline solution, and the titre of bacteriocin in units of activity (U) was determined by the spot test on phosphate buffered BHA seeded with the indicator strain *E. faecalis* S-47.

#### **Bacteriocin Treatments**

Cantaloupe melon (*Cucumis melo* L. var. *cantalupensis*) and watermelon (*Citrullus lanatus* var. *lanatus*) was purchased from local supermarkets. Juices were prepared from peeled cantaloupe and watermelon with a Moulinex Frutti Pro (Moulinex, France) fruit juice extractor. All manipulations were carried out under aseptic conditions.

The pH of juice (5.12 for cantaloupe and 5.97 for watermelon juices) was measured with a pH meter (Crison). Juices were inoculated 0.1% vol/vol with the ten-fold diluted *L. monocytogenes* inoculum. The challenged juices were supplemented with bacteriocin concentrates at 0, 100 and 200 U/ml and stored at 6 °C for 7 days.

Viable *Listeria* counts were determined by plating serial dilutions of samples on PALCAM agar with added supplement (Panreac, Barcelona, Spain). All assays were carried out in duplicate.

## **RESULTS AND DISCUSSION**

Viable *Listeria* counts of controls without bacteriocin in cantaloupe melon slightly increased by approx. 0.6 log cycles after 7 days of refrigeration storage (Table 1).

The antibacterial effect of bacteriocins was shown to be dependent on the bacteriocin preparation and its final concentration in the juice.

Enterocin EJ97 was the most effective among the three bacteriocins tested, achieving a gradual reduction of viable counts during storage when added at 100 U/ml. At 200 U/ml, EJ97 reduced viable counts of *L. monocytogenes* by 2.7 log cycles at day 1 of refrigeration storage and below detectable levels at days 5 and 7 of incubation (Table 1).

When tested in the watermelon juice (Table 2), addition of enterocin EJ97 at 200 U/ml reduced viable counts below detectable levels after day 3 of storage.

These results suggest that enterocin EJ97 could also be used as a hurdle against *L. monocytogenes* proliferation in refrigerated cantaloupe provided that initial contamination was not high as would be expected from a cross contamination incident.

Enterocin EJ97 has previously shown to have a strong anti-*Listeria* activity when tested in BHI broth at 30 AU/ml (García *et al.*, 2004).

However, the efficacy of bacteriocins in food systems could decrease due to the protective effect of food matrix.

**Research Article**

**Table 1: Effect of Enterocins AS-48, EJ97 and Seafood Bacteriocins SFB1 and SFB2 on the Viability of *L. Monocytogenes* in Cantaloupe Juice Stored at 6°C**

Treatment	Viable Counts (Log <sub>10</sub> CFU/ml at Different Storage Times (Days))				
	0	1	3	5	7
Control	5.67	5.47	5.83	6.11	6.29
EJ97, 100 U	5.74	4.87	2.34	2.44	1.67
EJ97, 200 U	5.22	2.97	1.23	<1.0	<1.0
SFB1, 100 U	5.54	5.01	4.67	3.78	3.62
SFB1, 200 U	5.2	4.78	3.87	2.12	2.25
SFB2, 100 U	5.47	4.97	4.14	3.23	3.14
SFB2, 200 U	4.97	4.6	2.97	2.34	2.05

**Table 2: Effect of Enterocins EJ97 and Fish Bacteriocins SFB1 and SFB2 on the Viability of *L. Monocytogenes* in Watermelon Juice Stored at 6°C**

Treatment	Viable Counts (Log <sub>10</sub> CFU/ml at Different Storage Times (Days))				
	0	1	3	5	7
Control	5.67	5.34	5.12	4.87	4.97
EJ97, 100 U	5.56	4.87	1.97	2.13	2.44
EJ97, 200 U	5.05	2.44	<1.0	<1.0	<1.0
SFB1, 100 U	5.49	5.11	4.44	4.02	3.97
SFB1, 200 U	5.17	4.97	3.67	2.56	2.44
SFB2, 100 U	5.54	5.37	4.67	3.97	3.63
SFB2, 200 U	4.88	4.47	3.34	2.97	2.14

Bacteriocins produced by enterococcal strains from seafood showed a more limited anti-*Listeria* effect in cantaloupe melon (Table 1). Yet, the tested bacteriocin preparations reduced initial viable counts by a maximum of 3.55 log cycles at day 5 for SFB1 preparation and by approx. 3.62 log cycles at day 7 for SB2, when both preparations were tested at 200 U/ml.

In the watermelon juice supplemented with seafood enterocins at 200 U/ml, maximum reductions of viable counts (3.23 to 3.53 log cycles) were observed at day 7 of storage (Table 2). The two bacteriocin preparations had similar effect in the sense that they gradually and slowly reduced viable counts during storage, while the effect of enterocin EJ97 as measured by decrease of viable counts was observed much faster.

**Concluding Remarks**

In conclusion, enterocin EJ97 performed better than enterocins from seafood enterococci in reducing viable counts of *L. monocytogenes*. The observed effects were similar regardless of the substrate being used (cantaloupe juice or watermelon juice). Therefore, enterococcal bacteriocins and in particular enterocin EJ97 could potentially be a candidate for controlling *L. monocytogenes* in melon juices.

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**Research Article**

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