Food Microbiology 48 (2015) 109–115

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Short communication

Antilisterial activity of bacteriocinogenic *Pediococcus acidilactici* HA6111-2 and *Lactobacillus plantarum* ESB 202 grown under pH and osmotic stress conditions

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ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form
3 November 2014
Accepted 25 November 2014
Available online 24 December 2014

Keywords:
Pediococcus acidilactici
Lactobacillus plantarum
Bacteriocin
Stress conditions
Bio-preservatives
Listeria spp

ABSTRACT

Bacteriocin producing lactic acid bacteria (LAB) cultures can be used as biopreservatives in fermented food products; thus the food industry is interested in stable cultures that produce bacteriocins consistently. Inhibtion of *Listeria* spp. by bacteriocinogenic *Pediococcus acidilactici* and *Lactobacillus plantarum* (both isolated from fermented meats) was investigated under conditions of stress induced by low pH and high salt concentrations. *Listeria monocytogenes* serogroup IIb (from cheese), *L. monocytogenes* serogroup IVb (from cheese), *L. monocytogenes* serogroup IIb (from ground beef) and *Listeria innocua* NCTC 11288 were used as target strains. *P. acidilactici* and *Lb. plantarum* demonstrated antilisterial activity under the stress conditions investigated (pH 3.5; pH 8.5; 7.5% NaCl). However, activity was dependent on the stress conditions applied and on the target organism. *L. monocytogenes* serogroup IIb (from ground beef) and *L. innocua* C 11288 were, respectively the most sensitive and the most resistant to the cell-free supernatants produced by the LAB investigated.

1. Introduction

Potential applications of bacteriocins produced by lactic acid bacteria (LAB) -*Lactobacillus* spp., *Leuconostoc* spp., *Lactococcus* spp., *Pediococcus* spp.—by the food industry have been extensively investigated (Balciunas et al., 2013; Callewaert et al., 2000; Devi and Halami, 2011; Kouakou et al., 2010; Mills et al., 2011; Työppönen et al., 2002). Several antilisterial bacteriocins from *Lactobacillus plantarum* (Martinez et al., 2013; Powell et al., 2007; Xie et al., 2011; Zacharof and Lovitt, 2012) and *Pediococcus acidilactici* (Albano et al., 2007; Altuntas et al., 2010) have been described and characterized in the last years. Pediocin PA-1/AcH (Devi and Halami, 2011), pediocin SA-1 (Anastasiadou et al., 2008), plantaricin ASM1 (Hata et al., 2010), plantaricin S (Martinez et al., 2013) and plantaricin MG (Gong et al., 2010) are heat stable and show stability at wide range of pH values. This stability potentiates their application in the food industry as biopreservatives. Työppönen et al. (2002) successfully used *Lb. plantarum*—as starter culture—to inhibit *Listeria monocytogenes* in dry sausages. *L. monocytogenes* was not detected during the ripening process. Kouakou et al. (2010) studied the effect of *P. acidilactici* in *L. monocytogenes*-seeded raw pork meat. Populations of *L. monocytogenes* were initially reduced, but repair of *L. monocytogenes* was observed after one week.

Increasing consumer demand for less processed and traditional food products has led to the use of biopreservatives to inhibit spoilage and pathogenic microorganisms (Balciunas et al., 2013). Bacteriocin producing LAB can be used as biopreservatives in starter or adjuvant cultures in food fermentations (Cui et al., 2012; Ghanbari et al., 2013; Jiang et al., 2012). Consequently, the food industry is interested in stable cultures that retain the ability to produce bacteriocins under a range of stresses that may be encountered in food products.

Several reviews have addressed the responses of LAB to environmental stresses (Champomier-Vergés et al., 2010; Van de Guchte et al., 2002). Most previous publications deal with gene expression in LAB under different environmental conditions (De Angelis et al., 2001; Fadda et al., 2010; Lemos et al., 2001). To our knowledge there have been few attempts to examine the

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http://dx.doi.org/10.1016/j.fm.2014.11.015
0740-0020/

production of bacteriocins by *Lb. plantarum* and *P. acidilactici* during growth under stressful conditions. Production of the bacteriocin amylovorin by *Lactobacillus amylovorus* in MRS broth was stimulated by stressful conditions, resulting in lower growth rates and increased activity against *Lactobacillus delbrueckii* subsp. *bulgaricus* (De Vuyst et al., 1996). Neysens et al. (2003)—based on observations by De Vuyst et al. (1996)—studied the growth limits and bacteriocin production of *Lb. amylovorus* under different stresses (temperature, pH, NaCl) during sourdough fermentation. Stress was found to induce biphasic fermentation kinetics and moderate production of amylovorin.

Bacteriocin production by LAB strains and their application as natural antimicrobials are well known (Balciunas et al., 2013). For industrial applications, it is important to know whether bacteriocins are produced under unfavorable environmental circumstances. Detailed examination of bacteriocin production under defined conditions in laboratory media is prerequisite to their consideration for use in food products. The latter should serve to clarify reasons for low bacteriocin production that are often reported when bacteriocin-producing strains are added to food matrices (Kouakou et al., 2010). To understand this phenomenon we studied the capacity of *P. acidilactici* HA-6111-2 and *Lb. plantarum* ESB 202 to inhibit *L. monocytogenes* and non-pathogenic *Listeria innocua* when grown in a laboratory culture medium under controlled stress conditions. These strains have been shown to produce antilisterial bacteriocins (Albano et al., 2007; Todorov et al., 2010).

2. Materials and methods

2.1. Origin of bacterial isolates

Bacteriocinogenic *P. acidilactici* HA-6111-2 (Albano et al., 2007) and *Lb. plantarum* ESB 202 (gently supplied by Dr Slavi D. Todorov; Todorov et al., 2010), both previously isolated from fermented meat sausages and deposited in the culture collection of Escola Superior de Biotecnologia (ESB), were selected for this study.

Three isolates of *L. monocytogenes* from the culture collection of the *Listeria* Research Center of ESB (LRCESB) (1486/1, serogroup IIb, isolated from cheese; 1604/2, serogroup IVb, isolated from cheese; 971, serogroup IIb, isolated from ground beef) and *L. innocua* NCTC 11288 were selected as target strains.

2.2. Growth and storage conditions

Lactic acid bacteria were cultured in de Man, Rogosa and Sharpe (MRS) broth (Biokar) at 37 °C for 18–22 h; *Listeria* spp. were grown in Tryptone Soy Broth (TSB; Biokar) supplemented with 0.6% (w/v) of yeast extract (LabM) (TSBYE) at 37 °C for 18–22 h. All strains were stored at −20 °C in appropriate culture media containing of 15% (v/v) glycerol. All bacterial strains were subcultured twice under appropriate conditions before use in experiments.

2.3. Preliminary examination of growth under different stress conditions

The effect of pH and salt concentration on the growth of each LAB was examined in preliminary trials to establish threshold conditions for further experimentation. Growth of each strain was examined in MRS broth at 30 °C adjusted to different pH with 10% HCl or 1 M NaOH (3.5, 4.0, 4.5, 5.0, 8.0, 8.5, 9.0, 9.5) and different NaCl concentrations (2.5, 5.0, 7.5 10.0%).

Table 1 shows the different pH and NaCl concentrations tested and the results obtained by visual inspection of turbidity where "+" denoted weak growth; "++", moderate growth, and "+++" good growth.

Table 1
Growth of *P. acidilactici* HA6111-2 and *Lb. plantarum* ESB 202 under different environmental conditions after 48 h.

	pH							
	3.5	4	4.5	5	8	8.5	9	9.5
P. acidilactici HA6111-2	++[a]	++	+++	+++	+++	++[a]	+	No growth
Lb. plantarum ESB 202	++[a]	++	+++	+++	+++	++[a]	+	No growth

	NaCl concentration			
	2.5%	5%	7.5%	10%
P. acidilactici HA6111-2	+	++	++[a]	No growth
Lb. plantarum ESB 202	+++	++	++[a]	No growth

+: Weak growth.
++: Moderate growth.
+++: Good growth.
[a] Selected conditions.

The most extreme stress conditions where moderate growth was observed after 48 h were selected for further experiments.

2.4. Antilisterial activity during growth under stress conditions

Modified MRS broth (Table 1) was inoculated with 1% (v/v) of an overnight culture of each LAB and incubated at 30 °C for 48 h. Samples were collected every 3 h for 48 h and LAB were enumerated on MRS agar incubated at 30 °C for 24 h. Changes in pH and optical density at 600 nm were also recorded every hour for 48 h. Antilisterial activity of neutralized cell-free supernatants (hereafter referred as supernatants) was measured every 3 h for 48 h, as described by Van Reenen et al. (1998). *L. monocytogenes* 1486/1, *L. monocytogenes* 1604/2, *L. monocytogenes* 971 and *L. innocua* NCTC 11288 were used as target strains.

Antilisterial activity measured in supernatants from cultures grown in MRS adjusted to pH 6.5 incubated at 30 °C was used as a control. All assays were carried out in duplicate.

Viable bacterial counts were expressed as log N and antilisterial activity was expressed as arbitrary units (AU) per ml. One AU is defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition. Standard deviations were calculated using Microsoft Excel, which was also used to plot the graphs in Figs. 1–7.

3. Results and discussion

Results of preliminary growth experiments under stress conditions are presented in Table 1. Antilisterial activity was examined where moderate growth was observed after 48 h under the most extreme stress conditions (pH 3.5, pH 8.5 and 7.5% NaCl; Table 1).

When *Lb. plantarum* was grown at 30 °C in MRS, the highest level of antilisterial activity (25,600 AU/mL) was recorded after 15 h against *L. monocytogenes* and after 18 h against *L. innocua*. For *P. acidilactici*, the same level of activity was recorded after 18 h against both *Listeria* species (Fig. 1).

Growth of *P. acidilactici* and *Lb. plantarum* at pH 8.5 and antilisterial activity are shown in Figs. 2 and 3, respectively. For both LAB, an extended lag phase was observed as well as a delay in antilisterial activity. LAB are able to produce hyaluronic acid instead of lactic acid under alkaline stress (Liu et al., 2008). In this way cells are able to decrease the pH to levels appropriate for growth. This can be an explanation for the adaptation of *P. acidilactici* and *Lb. plantarum* to the imposed alkaline stress.

The maximum level of activity against *L. monocytogenes* strains and *L. innocua* was 25,600 AU/mL for *P. acidilactici* and 25,600 AU/mL and 12,800 AU/mL, respectively, against *L. monocytogenes* strains and *L. innocua*, for *Lb. plantarum*. Maximum antilisterial