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Phenotypic comparison and DNA sequencing analysis of a wild-type and a pediocin-resistant mutant of *Listeria ivanovii*

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Abstract

Listeria ivanovii is one of the two pathogenic species within the genus *Listeria*, the other being *L. monocytogenes*. In this study, we generated a stable pediocin resistant mutant Liv-r1 of a *L. ivanovii* strain, compared phenotypic differences between the wild-type and the mutant, localised the pediocin-induced mutations in the chromosome, and analysed the mechanisms behind the bacteriocin resistance. In addition to pediocin resistance, Liv-r1 was also less sensitive to nisin. The growth of Liv-r1 was significantly reduced with glucose and mannose, but less with cellobiose. The cells of Liv-r1 adsorbed less pediocin than the wild-type cells. Consequently, with less pediocin on the cell surface, the mutant was also less leaky, as shown as the release of intracellular lactate dehydrogenase to the supernatant. The surface of the mutant cells was more hydrophobic than that of the wild-type. Whole genome sequencing revealed numerous changes in the Liv-r1 chromosome. The mutations were found e.g., in genes encoding sigma-54-dependent transcription regulator and internalin B, as well as in genes involved in metabolism of carbohydrates such as glucose and cellobiose. Genetic differences observed in the mutant may be responsible for resistance to pediocin but no direct evidence is provided.

Keywords: Bacteriocin; *Listeria ivanovii*; Resistance; Antimicrobial activity

1. Introduction

Lactic acid bacteria (LAB) are a diverse group of Gram positive, catalase negative, and oxidase negative bacteria with the GRAS (generally recognized as safe) status. LAB are widely used as starters in food fermentations, but also as protective cultures against foodborne pathogens and spoilage organisms, because they often produce antimicrobial compounds such as bacteriocins. Most bacteriocins of LAB are small, heat-stable, cationic antimicrobial peptides which can be classified into two main groups: post-translationally modified (class I) and non-modified (class II) bacteriocins [1]. These classes can then be divided into several subclasses, mainly based on their structures. Besides the class Ia bacteriocin nisin, which is used as a food preservative, also class IIa (a.k.a. pediocin-like) bacteriocins are considered promising candidates for industrial applications [2]. They have gained interest because of their strong inhibitory effect on *Listeria* sp. More than 40 class IIa bacteriocins have been identified and sequenced including pediocin, sakacin P, leucocin C, enterocin A etc [3, 4]. With the consumers' increasing demand for natural and minimally processed food, as well as continuing emergence of antibiotic resistant bacteria, it has been suggested that class IIa bacteriocins could have applications as antimicrobials in food industry as natural preservatives, and in human and veterinary medicine as alternatives to antibiotics [5]. Even though a few fermentation products based on pediocin have been commercialized, bacteriocins are still underutilized by the food industry, at least partly due to insufficient knowledge about how these antimicrobials work [6-8].

Positively charged class IIa bacteriocins easily bind to the negatively charged bacterial cell surface, penetrate into the hydrophobic part of the cell membrane, and cause bacterial lysis by forming pores [9]. The major uptake system for glucose and mannose, the mannose phosphotransferase system (Man-PTS), and particularly its membrane proteins IIC and IID form a receptor for class IIa bacteriocins [10]. By binding and inserting into Man-PTS, class

IIa bacteriocins form pores in cell membrane, which makes cells leaky, and eventually leads to cell death. Although resistance against class IIa bacteriocins does not happen at high frequency in nature, it has been reported to be developed in the laboratory, mainly by downregulation of Man-PTS gene expression [11]. Ramnath et al. [12] and Xue et al. [13] also demonstrated that there was a link between the expression of Man-PTS and resistance to class IIa bacteriocins. In addition, structural changes in cell surface have been proposed to be involved in the bacteriocin resistance [14].

Previous studies of class IIa bacteriocin resistance have mainly been focused on *Listeria monocytogenes*, a foodborne human pathogen causing disease outbreaks and food recalls [15]. The objective of this study was to examine the pediocin resistance in *Listeria ivanovii*, a pediocin highly sensitive strain and also of great economic importance as pathogen in livestock [16]. Cells were made resistant by pediocin challenge, and the changes such as cross resistance to antimicrobials, metabolic and surface properties, and chromosomal mutations were investigated and compared with the wild-type strain. Understanding the mechanism behind the bacteriocin resistance in different bacteria helps elucidate the bacteriocin-cell interaction, which, in turn, may be useful for bacteriocin applications.

2. Materials and Methods

2.1. Bacterial strains and growth conditions

L. ivanovii wild-type strain DSMZ 20750 (Liv) and resistant variant (Liv-r1) were cultured in Brain Heart Infusion (BHI) purchased from Beijing Aobox Bio-Tech Co., Ltd. (Beijing, China) at 37 °C. Three class IIa bacteriocin-producing strains used in this study were cultured as follows. *Pediococcus acidilactici* PA003 and *Lactobacillus plantarum* CICC 24194 were cultured at 37 °C in de Man, Rogosa and Sharpe broth (MRS; Beijing Aobox Bio-Tech Co., Ltd., Beijing, China), and *Lactobacillus curvatus* ATCC 51436 was grown in MRS broth at 28 °C. Tryptone Soy Broth (TSB; 15.5 g/L tryptone, 5 g/L soybean peptone, 6.5 g/L yeast extract, 5 g/L NaCl) was used for monitoring *L. ivanovii* growth in the presence of different sugars. For solid media, 2% (wt/vol) agar was added.

2.2. Bacteriocin activity assays

Pediocin was prepared from *P. acidilactici* PA003 according to the method of Wang et al. [17]. The neutral cell-free culture supernatants (nCFS) containing class IIa bacteriocins were prepared by centrifugation of cultures at 7, 000 g for 10 min, adjustment of supernatants to pH 7.0 and filtration through a 0.22 μm pore size filter (Millipore, US). Nisin was purchased from Zhejiang Silver-Elephant Bio-engineering Co., Ltd. (Taizhou, China). Bacteriocin activity was assayed by the agar well diffusion method and expressed in units [18]. The arbitrary units (AU) per milliliter were equal to $2^n \times (1000/x)$, where n is the number of wells showing clear inhibition of the indicator zone and x is the sample volume.

2.3. Generation of bacteriocin resistant variant and antimicrobial susceptibility test

L. ivanovii-derived strain resistant to pediocin was isolated by cultivation on plates containing gradually increasing pediocin concentration (50-200 AU/mL, determined according to section 2.2). The stability of the pediocin resistance of the isolate Liv-r1 was verified after 10 serial sub-culturings in the absence of pediocin. The susceptibility and

minimum inhibitory concentrations (MIC) for antibiotic agents including kanamycin and ampicillin against *L. ivanovii* strains were determined by agar well diffusion method, for determining the possibility of cross-resistance to antibiotics. The MIC was defined as the lowest concentration resulting in a clear inhibition zone.

2.4. Growth situations with different sugars

The growth of the strains Liv and Liv-r1 on different sugars was examined in TSB supplemented with 1% (wt/vol) glucose, mannose or cellobiose (Shanghai Yuanye Bio-Tech Co., Ltd., Shanghai, China). The media were inoculated with 1% of the overnight cultures, and the growth was monitored by measuring the optical density at 600 nm for 12 h with a spectrophotometer (Infinite 200, Tecan, US).

2.5. Adsorption of pediocin onto L. ivanovii *strains*

L. ivanovii cells were collected after overnight cultivation by centrifugation at 7, 000 g for 10 min. Pellets were washed with 5 mM phosphate buffer (pH 6.0) for 3 times and resuspended to 10^8 cfu/mL in the same buffer containing 640 AU/mL pediocin, determined according to section 2.2. The mixture was incubated at 30 °C for 1 h. Pediocin activity of the supernatant obtained after centrifugation at 7, 000 g for 10 min was determined. The phosphate buffer containing 640 AU/mL pediocin was used as a negative control.

2.6. Assessment of extracellular enzymes for pediocin inactivation

The CFSs of *L. ivanovii* cultures were obtained after overnight cultivation and centrifugation at 7, 000 g for 10 min. Pediocin was mixed with the CFSs and incubated at 30 °C for 0.5 and 1.5 h. Bacteriocin activity was assayed by agar well diffusion according to section 2.2. Pediocin mixed with BHI broth was used as a negative control.

2.7. Assessment of cell surface hydrophobicity

L. ivanovii cultures were centrifuged at 7, 000 g for 10 min. Cells were washed 3 times with 50 mM phosphate buffer (pH 6.5) and resuspended in the same buffer to OD_{600} of

approximately 0.5. Then, 4.8 mL of each bacterial suspension was mixed with 0.8 mL of xylene in a glass tube, and vigorously shaken for 1 min. After incubation at room temperature for 45 min, the aqueous phase was removed carefully and the OD_{600} was determined. The cell surface hydrophobicity was calculated with the following equation according to Pérez et al. [19]: Adherence (%) = (1- A/A_0) × 100, where A_0 and A are the OD_{600} of the bacterial suspension before and after mixing with xylene, respectively.

2.8. Measurement of membrane permeability using extracellular lactate dehydrogenase (LDH)

L. ivanovii strains were cultured 8 h and centrifuged at 11, 000 g for 10 min. Cell pellets were washed twice with 10 mM phosphate buffer (pH 7.2) and resuspended in the same buffer to the concentration of 10^8 cfu/mL. Then, the suspensions were mixed with 64 AU/mL pediocin, determined according to section 2.2 and incubated at 37 °C. Samples were taken out and filtered through a 0.22 μm pore size filter at 0, 1, 3 and 4 h. The filtrate was determined for extracellular LDH using a LDH kit (Jiancheng Biology Engineering Institute, Nanjing, China). *L. ivanovii* suspensions without pediocin treatment were used as controls.

2.9. DNA extraction and sequencing

Genomic DNA from wild-type *L. ivanovii* and the resistant variant was isolated from overnight cultures using standard cetyl trimethyl ammonium bromide (CTAB) method [20]. DNA samples were submitted to the Biomarker Technologies (Beijing, China) and sequenced using Illumina sequencer (NOVA seq) according to the instructions of the manufacturer.

2.10. Sequencing analysis

Pair-end reads from the sensitive wild-type strain and the corresponding resistant variant were subjected to quality evaluation and filtering before obtaining clean reads, and then compared to the fully annotated database reference genome of *L. ivanovii* (https://www.ncbi.nlm.nih.gov/genome/?term=Listeria+ivanovii) using Burrows-Wheeler

Alignment tool (BWA) software [21]. Observed differences (SNPs or indels) between the genomes of the two sequenced strains were analysed to identify the mutations possibly causing pediocin resistance.

2.11. Statistical analysis

Results were presented as mean ± standard deviation (SD) from three replicates. A one-way analysis of variance in SPSS software version 17 was performed for evaluation of each pair of strains, wild-type and pediocin-resistant mutant, based on post hoc analysis with significance level of $P < 0.05$.

3. Results and Discussion

3.1. Generation of pediocin resistant L. ivanovii

Pediocin resistant *L. ivanovii* cells were isolated after exposure to gradually increasing concentrations of pediocin at a frequency of 10^{-6}, consistent with reported class IIa bacteriocin resistance frequency in *Listeria* depending on the conditions and strains [22, 23]. One mutant of *L. ivanovii* with stable resistant phenotype, designated as Liv-r1, was chosen for comparisons with the wild-type *L. ivanovii*. Wild-type strain was sensitive to the nCFSs from three class IIa bacteriocin-producing strains, among which pediocin was the most effective bacteriocin (Table 1). The Liv-r1 mutant was resistant to all tested nCFSs showing no visible inhibition zones in agar well diffusion analysis. Besides, Liv-r1 displayed increased resistance to class I bacteriocin nisin but not to ampicillin (Table 1 and 2). Cross resistance among bacteriocins has been described in several reports. Pediocin 34 resistant mutant of *L. monocytogenes* showed cross resistance to enterocin FH99, and the nisin resistant *Enterococcus faecium* variant conferred cross resistance to both pediocin 34 and enterocin FH99 [24]. Likewise, in the study by Kumariya et al. [9], pediocin resistant *Enterococcus faecalis* was also resistant to nisin. However, there was no cross resistance to antibiotics in

Liv-r1, indicating that acquiring bacteriocin resistance in *Listeria* may not hinder the antibiotic therapy. In fact, it may render the cells even more sensitive to antibiotics due to fitness cost of developing bacteriocin resistant phenotype, as reported previously by Martínez and Rodríguez [25].

3.2. Carbohydrate utilization

Bacteria transport carbohydrates mainly by specific phosphoenolpyruvate-dependent phosphotransferase systems (PTS). In the genome sequence of *L. monocytogenes* EGDe, seven families of *pts* genes have been recognized (Glc-PTS, Man-PTS, Lac-PTS, Fru-PTS, Gut-PTS, Gat-PTS and Asc-PTS) [26, 27]. It has been reported that the class IIa bacteriocin resistant *L. monocytogenes* grow slower on mannose and glucose than on cellobiose, which indicates that the gene expression of the Man-PTS, the main receptor of class IIa bacteriocins, is downregulated in the resistant mutants [11]. Similarly, spontaneous pediocin resistant *E. faecalis* mutants have shown reduced glucose consumption [28]. Therefore, in the present study, the growth of *L. ivanovii* strains was evaluated on different carbohydrates, i.e., glucose, mannose, and cellobiose. When compared to the wild-type strain, the growth of the resistant variant Liv-r1 was reduced with all three carbohydrates (Fig. 1). However, like in the study by Tessema et al. [11] mentioned above, the variant grew remarkably better on cellobiose than on glucose and mannose (Fig. 1), suggesting similar mechanism of bacteriocin resistance in *L. ivanovii* and *L. monocytogenes*. According to Stoll and Goebel [27], cellobiose is transported by both Glc-PTS and Lac-PTS in *L. monocytogenes*, whereas glucose and mannose are transported by Glc-PTS and Man-PTS. Therefore, possible downregulation of Man-PTS would have smaller effect on growth with cellobiose. In addition, it has been shown that growth on glucose, mannose and fructose increased the sensitivity of *L. monocytogenes* to leucocin A or carnocyclin A, while growth on cellobiose and sucrose increase the resistance to bacteriocins [29]. In conclusion, it seems likely that carbohydrate metabolism plays a crucial

role in class IIa bacteriocin sensitivity in different bacterial species.

3.3. Adsorption of pediocin on Listeria *cells*

To test whether the pediocin resistance was based on poorer adsorption of the peptide onto cell surface, pediocin was mixed with wild-type and resistant variant cells, and the bacteriocin activity was measured from the supernatant after 1 h incubation in a buffer with pH 6.0. Pediocin has been shown to adsorb onto cell surface in a pH-dependent manner, the strongest adsorption occurring around at pH 6.0 [30]. As shown in Table 3, the residual concentrations of free pediocin in PBS decreased both in wild-type and resistant cells, indicating that the pediocin had adsorbed on the cell surface. However, the adsorption level of the wild-type cells was twice as much of that of the resistant variant, suggesting that there may be less specific receptor sites on the surface of Liv-r1, or that the cell surface of Liv-r1 may have changed somehow, making it less adherent to pediocin. One way or another, less pediocin adsorbed on the variant *Listeria* cells, which partly reduces the antimicrobial activity for taking effect.

3.4. Examination of pediocin inactivation by extracellular enzymes

Many bacteria, e.g., *Bacillus subtilis* and *Lactococcus lactis*, produce extracellular proteases, which can degrade antimicrobial peptides [15, 31]. Even though this has never been shown to happen with *Listeria* and class IIa bacteriocins, the secretion of proteases or other bacteriocin-inactivating enzymes is a possible mechanism for resistance, and should not be excluded without testing it. Therefore, to examine whether the resistant mutant excretes a pediocin-inactivating enzyme, the bacteriocin was mixed and incubated in *Listeria* CFSs, and the pediocin activity was determined. The results shown in Table 4 verified that neither the wild-type, nor the pediocin resistant *Listeria* had any pediocin-degrading activity. This result further supports the previous finding that pediocin resistance of Liv-r1 was, at least partly, mediated by reduced pediocin adsorption onto the cell surface, and not by secretion of proteases.

3.5. Pediocin-induced cell leakage

Cell leakage caused by pore-forming activity of pediocin was evaluated by measuring LDH enzyme in culture supernatants. The culture supernatant of the wild-type *L. ivanovii* had significantly higher LDH activity in the presence of pediocin compared with that of Liv-r1 at the same time (Fig. 2). Immediately after pediocin addition (time point zero), the wild-type cells started to leak, and the leaking continued through the monitored four hours. On the contrary, in Liv-r1 supernatants at time point zero, the LDH activity with and without pediocin was approximately the same. During the 4-h test, the LDH activity in the Liv-r1 supernatant with pediocin still increased, but evidently less than in wild-type supernatant. The results demonstrated that pediocin took effect in a rapid manner. Similar fast lytic effect of pediocin has also been reported before. In 30 min after pediocin treatment, *Listeria* cells were visibly leaking in SEM image [32]. Not surprising, the results also showed that the resistant cells were more tolerant than the wild ones. The resistance could be explained by alterations of cell surface properties either by decreased fluidity, increased rigidity or decreased negative charges disrupting pediocin-receptor interaction [33].

3.6. Cell surface hydrophobicity

It has been previously shown that there are several mechanisms of class IIa bacteriocin resistance, one of which is related to alterations in cell membrane or cell wall [34]. In this study, we compared the hydrophobicity of cell surfaces of wild-type and the Liv-r1 mutant by determining the cells' adhesion to xylene. Significantly higher cell surface hydrophobicity ($P < 0.05$) was seen in resistant *L. ivanovii* variant compared with the wild-type (Fig. 3). In Kumariya et al. [9] work, the increase in cell surface hydrophobicity was collinear with the degree of pediocin resistance. In addition, Lather et al. [35] have also pointed out the contribution of cell surface hydrophobicity as an adaptive reaction against antimicrobial agents. As the bacterial cell envelope is negatively charged, the increased hydrophobicity may

suggest less negative charges on the surface, which decreases the interaction with cationic antimicrobial peptides, leading to increased resistance.

3.7. Whole-genome sequencing

The technological progress of whole-genome sequencing (WGS) may grant access to potential mechanisms of bacteriocin-resistance by providing high-throughput information at bacterial molecular level. In this work, to find out which genes actually had been mutated in the pediocin-resistant mutant Liv-r1, the genomes of the mutant and the wild-type strain were sequenced. After filtering, 4463819 and 4802008 clean reads were obtained from Liv and Liv-r1, respectively. The GC content of Liv was 37.14% with sufficient quantity (Q20 = 98.12%) and quality (Q30 = 94.26%) of the data for further analysis. The GC content of Liv-r1 was 37.12% with sufficient quantity (Q20 = 98.04%) and quality (Q30 = 94.09%) of the data.

The genome sequences of the pediocin-resistant strain showed 12 single nucleotide polymorphisms (SNP) compared to the wild-type reference, 6 of which belonged to non-synonymous coding, 4 to synonymous coding and 2 to upstream regions of genes. Precise locations of these SNPs, and the annotation results of predicted proteins in NR (non-redundant) and Swiss-Prot databases are shown in Table 5. Three SNPs were related to genes *i-inlB1* and *i-inlB2* (gene IDs 1383 and 503 in Table 5), encoding variants of internalin B, surface proteins involved in invasion of multiple mammalian cell types in *L. monocytogenes* by activating junctional endocytosis [36]. Two mutations in the *i-inlB1* gene caused amino acid changes (V5631 and I985V) in the GW-domains responsible for non-covalent binding of the i-InlB1 internalin onto the bacterial cell surface. However unlikely, it cannot be excluded that these two amino acid changes have had an effect on i-InlB1 protein and its binding or release to/from the cell surface. Winkelströter et al. [37] demonstrated that in the presence of bacteriocins from *Lactobacillus sakei*, *Leuconostoc mesenteroides*, and *E. faecium*, the

299 expression of the internalin A gene *inlA* was downregulated in *L. monocytogenes*.
300 Downregulation of internalins is likely to reduce the virulence of the strain. However, in our
301 study, the observed mutation related to the gene *i-inlB2* was quite far, 1289 bp upstream of
302 the gene, and thus probably did not have much effect on the expression level of the *i-inlB2*
303 gene. As cell surface proteins, internalins affect the cell's surface properties, and thus
304 mutations or changes in their expression level may also affect the binding or adhesion of
305 bacteriocins onto cell surface. However, the effect of the observed SNPs in the protein
306 properties or gene expression levels were not determined in this work, and therefore further
307 studies would be needed to clarify which SNPs, if any, actually had effect on the pediocin
308 resistant phenotype.

309 Altogether 87 indels were found in the resistant mutant Liv-r1, including frameshifts,
310 upstream and downstream mutations, codon deletions and codon deletion plus codon insertion
311 (Supplemental material). The most common mutation was frameshift. Annotation analysis
312 was conducted in COG database, and in total 10 indels were found to belong to carbohydrate
313 transport and metabolism, 9 were involved in transcription, 6 in inorganic ion transport and
314 metabolism, and 5 in amino acid transport and metabolism. Metabolism of carbohydrates
315 including glucose, cellobiose, lactose, and β-glucoside were identified in the indels (Table 6).
316 These results corresponded with the observed differences in carbohydrates metabolism of
317 wild-type and variant one. Laursen et al. [38] have previously pointed out that after exposure
318 to 180 min to pediocin-containing *Lb. plantarum* WHE92 supernatant, 25 genes related to
319 carbohydrate transport and metabolism were upregulated, while 31 related genes were
320 downregulated in *L. monocytogenes*. Thus, class IIa bacteriocin sensitivity may be associated
321 with genes in charge of carbohydrate transportation, metabolism and regulation.

322 Regarding the transcription class of the observed indels, gene 910 annotated as sigma-54-
323 dependent transcriptional regulator was found to have a frameshift. Sigma-54 is in charge of

regulating numerous genes, often related to sugar transport and metabolism, including Man-PTS [39]. It has also been recognized to have a role in the resistance of class IIa bacteriocins in *E. faecalis* and *L. monocytogenes* [40, 41]. In a study about class IIa bacteriocin resistance in *Enterococcus* by Geldart and Kaznessis [42], a mutation in the sigma-54 transcription factor and the disruption of the sigma-54-associated activator protein, ManR, were found after genome comparison.

4. Conclusion

This study provides the characterisation of class IIa bacteriocin resistance in *L. ivanovii*. The resistant cells exhibited reduced growth on glucose, adsorption of pediocin, and cell lysis by pediocin attack. Additionally, increased cell surface hydrophobicity was detected in resistant mutant compared with the wild-type. Lastly, results from whole-genome sequencing provided evidence to suggest the vital role of the carbohydrate transportation, metabolism and regulation in the development of pediocin resistance. PTS systems responsible for different carbon sources will be further examined for their functions in *L. ivanovii* for in-depth understanding of bacteriocin-cell surface interaction.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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462 **Legends to figures**

463 Fig.1. Growth situations of the wild-type Liv (A) and the pediocin resistant variant Liv-r1 (B)
464 in TSB broth containing glucose, mannose, or cellobiose. The growth of the variant strain
465 Liv-r1 is reduced stronger on glucose and mannose than on cellobiose.

466 Fig.2. LDH activity at different time points after pediocin addition. High LDH activity in the
467 wild-type strain Liv with pediocin indicates strong and fast cell leakage due to pore-forming
468 activity of pediocin. Cells of the resistant variant Liv-r1 leaked noticeably less, as seen as
469 lower LDH activity. Statistically significant difference between Liv and Liv-r1 was found (*P
470 < 0.05).

471 Fig.3. Cell surface hydrophobicity of the wild-type Liv and the pediocin-resistant variant Liv-
472 r1. The resistant variant showed higher cell surface hydrophobicity than the wild-type strain.
473 Statistically significant difference between Liv and Liv-r1 was found (*$P < 0.05$).

Table 1 Activities of neutral cell-free culture supernatants (nCFS) from class IIa bacteriocin-producing strains and nisin to *Listeria* strains.

Strain	*P. acidilactici* PA003 (AU/mL)	*Lb. curvatus* ATCC 51436 (AU/mL)	*Lb. plantarum* CICC 24194 (AU/mL)	Nisin (AU/mL)
Liv	640	320	320	1280
Liv-r1	No inhibition	No inhibition	No inhibition	320

Table 2 The minimum inhibitory concentrations (MICs) of antibiotics.

Strain	MIC of kanamycin (μg/mL)	MIC of ampicillin (μg/mL)
Liv	12.5	12.5
Liv-r1	6.3	12.5

Table 3 Pediocin activities in PBS (pH 6.0) after incubation with *Listeria* strains. PBS containing 640 AU/ml pediocin without cells was used as a control.

	Control	Liv	Liv-r1
Pediocin activity (AU/ml)	640	160	320

12 Table 4 Pediocin activities (AU/mL) after mixing with *Listeria* CFSs at different times. Pediocin

13 mixed with BHI broth was used as a control.

Time	Control	Liv	Liv-r1
0.5 h	320	320	320
1.5 h	320	320	320

14

15 Table 5 Annotation of SNP with non-synonymous coding and upstream types.

Effect	Gene ID	Site in gene	NR annotation	Swissprot annotation
Non-synonymous coding	281	526	peptidase M4 family protein	Zinc metalloproteinase
	469	759	hypothetical protein	UPF0365 protein
	519	890	hypothetical protein	--
	1383	1687	GW domain-containing glycosaminoglycan-binding protein	Internalin B
	1383	2953	GW domain-containing glycosaminoglycan-binding protein	Internalin B
	2288	57	hypothetical protein	--
Upstream	503	-1289	GW domain-containing glycosaminoglycan-binding protein	Internalin B
	658	-97	HdeD family acid-resistance protein	--

16

17 Table 6 Summary of small indels annotated in genes involved in carbohydrate transport and

18 metabolism.

Effect	Gene ID	Site in gene	NR annotation	Swissprot annotation
Frameshift	254	368	glucose transporter GlcU	Putative sugar uptake protein lin0215
	260	2958	glycoside hydrolase family 31 protein	Alpha-xylosidase
	261	2159	Alpha-glucosidase 2	glycoside hydrolase family 31 protein
	436	944	PTS fructose transporter subunit IIBC	PTS system fructose-specific EIIB
	558	184	Uncharacterized ABC transporter extracellular-binding protein YurO	ABC transporter substrate-binding protein

	2223	923	--	DUF3502 domain-containing protein
	2225	633	Uncharacterized multiple-sugar transport system permease YteP	protein LplB
	2942	102	Gluconokinase	gluconate kinase
Upstream	63	-52	PTS beta-glucoside transporter subunit EIIBCA	PTS system beta-glucoside-specific EIIBCA component
Downstream	2552	1287	Uncharacterized MFS-type transporter YuxJ	MFS transporter

Fig. 1A

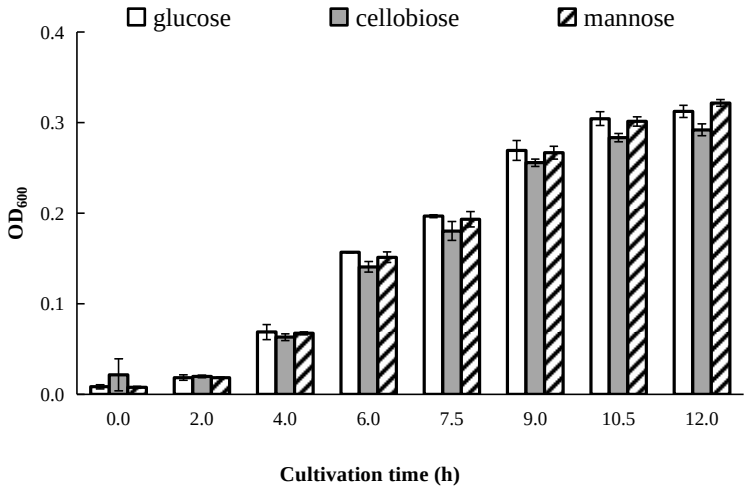

Fig. 1B

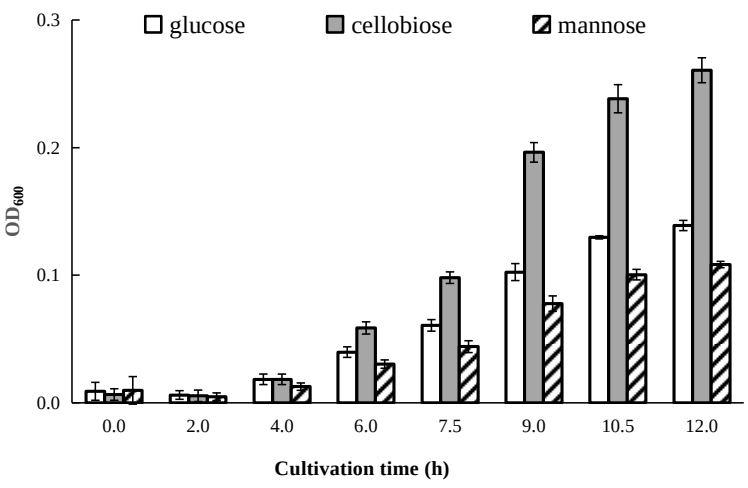

Fig. 2

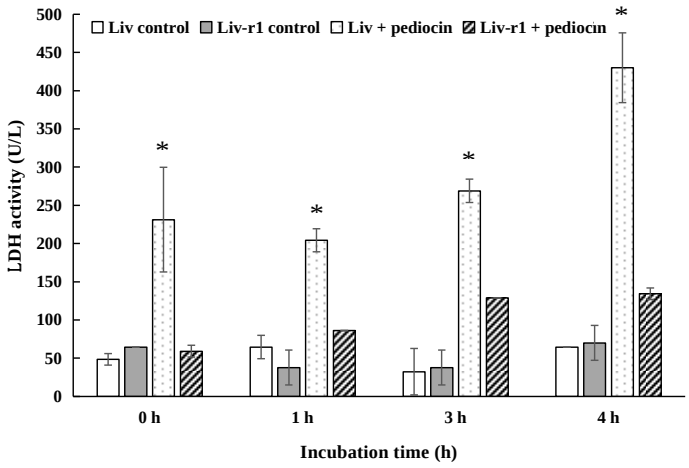

Fig. 3