INTRODUCTION

It is of great importance to control the foodborne pathogen Listeria monocytogenes (L. monocytogenes) on food safety perspective, and also for human health (Gray & Killinger, 1966; Lebreton, Stavru, & Cossart, 2015; Radoshevich & Cossart, 2018; Swaminathan & Gerner-Smidt, 2007). L. monocytogenes, a member of the genus Listeria, is widely distributed in agricultural environments, such as soil, manure, water, and plants (Gandhi & Chikindas, 2007; Smith, Moorhouse, Monaghan, Taylor, & Singleton, 2018). Foods like milk (Verraes et al., 2015), meat products (Alvarez Ordonez, Leong, Hickey, Beaufort, & Jordan, 2015), vegetables (Valimaa, Tilsala-Timirjärv, & Virtanen, 2015), processed ready-to-eat and cold-stored meat, and dairy products are considered high-risk foods for L. monocytogenes infections (Tompkin, 2002). This organism is a recognized foodborne pathogen that causes many diseases, from...
mild gastroenteritis to severe blood and/or central nervous system infections, as well as abortion in pregnant women (Vazquez-Boland et al., 2001). The lethal rate of listeriosis is more than 25% (Pamer, 2004). In addition, \textit{L. monocytogenes} was recently reported to have the ability to form biofilm, which brought a bigger problem to food processing (Giaouris et al., 2015; Mathur et al., 2017). Therefore, it is of vital practical significance to inhibit the growth of \textit{Listeria} and control its contamination in foods to ensure the quality and safety of food.

Bacteriocins are antimicrobial proteins or polypeptides synthesized by bacterial ribosomes and secreted to extracellular (Bastos, Coelho, & Santos, 2015; Vanderwal, Luirink, & Oudega, 1995). Recent studies have shown that bacteriocin can be degraded in human body, nontoxic, and target bacteria are not easy to produce resistance (Franz et al., 2018; Mathur et al., 2017). Besides nisin, bacteriocins produced by lactic acid bacteria (LAB) are attracting considerable interest for use as alternative food preservatives (Favaro, Penna, & Todorov, 2015), especially the class IIa bacteriocins that is well recognized for their high antilisterial activity. However, the target cell recognition mechanism of class IIa bacteriocins is poorly understood.

Previous research suggested that mannose phosphotransferase system (Man-PTS) functions in the regulation of various bacterial physiological processes (Postma, Lengeler, & Jacobson, 1993; Reizer et al., 1998; Saier, 1989). The Man-PTS is consisted by four components named IIA, IIB, IIC, and IID. It was reported that there was a putative Man-PTS IIAB component in a leucocin A-resistant strain of \textit{L. monocytogenes} (Ramnath, Beukes, Tamura, & Hastings, 2000). Also, interruption of either the proximal (mptA) or distal (mptD) gene in \textit{Enterococcus faecalis} resulted in resistant to mesentericin Y105 (Karine et al., 2001). Thus, the subclass IIa bacteriocin is likely to use a Man-PTS component as specific target (Hechard & Sahl, 2002). Furthermore, sensitivity to class IIa bacteriocins of lactic acid bacteria was recently associated with Man-PTS permease in \textit{L. monocytogenes}, and it shows that expression of mptC alone is sufficient to confer sensitivity to class IIa bacteriocins in \textit{Lactococcus lactis} (Ramnath, Arous, Gravesen, Hastings, & Hechard, 2004). The antibacterial mechanism of lactococcin A, a class IIa bacteriocin, on target bacteria was described in detail by Diep. Skaugen, Salehian, Holo, and Nes, (2007). Kjos, Nes, and Diep, (2009) pointed that the level of bacteriocin susceptibility for a bacterial is primarily determined by differences in its Man-PTS proteins, although the expression levels of the corresponding genes also play an important role. The results of pediocin PA-1 (Kjos, Nes, & Diep, 2011; Opsata, Nes, & Holo, 2010; Zhou, Wang, Wang, Ren, & Hao, 2016), pediocin-like bacteriocin (Colombo, Chalon, Navarro, & Bellomio, 2018), and garvicins A, B, and C also support this conclusion.

Durancin GL, a new bacteriocin produced by \textit{Enterococcus durans} 41D, was found to have high antilisterial activity (Du, Somkuti, Renye, & Huo, 2012). By alanine-scanning mutational analysis with site-directed mutagenesis, it showed that durancin GL residues were important for antimicrobial activity and specificity, such as three mutations lost their antimicrobial activity, 10 mutations demonstrated a decreased effect on the activity, and seven mutations exhibited relatively high activity; besides, four mutants demonstrated a narrower antimicrobial spectrum than wild-type durancin GL, and another four mutants displayed a broader target cell spectrum and increased potency relative to wild-type durancin GL (Ju et al., 2015). Study on phenotypic and genotypic alterations of durancin GL-resistant enterococcus durans strains showed that durancin GL can cause damage to bacterial cells of wild bacteria and the increased unsaturated fatty acid and decreased mannose phosphotransferase system gene expression in resistant strains could contribute to durancin GL resistance (Du, Liu, Liu, Ju, & Yuan, 2016). These findings broaden our understanding antimicrobial activity of durancin GL; however, the related mechanism is not clear yet, and this study focused on clarifying the targeting inhibition of \textit{L. monocytogenes} by durancin GL.

2 MATERIALS AND METHODS

2.1 Bacterial strain and culture condition

Bacterial strains, plasmids, and primers used in this study are listed in Table 1. \textit{L. monocytogenes} Scott A was provided by Jiangsu provincial center for disease control and prevention in Nanjing, China. \textit{L. monocytogenes} was propagated in brain heart infusion (BHI) medium (Land Bridge Co. Ltd) under aerobic conditions at 37°C and 200 rpm in a shaking incubator. A single colony of yeast NMY51 was inoculated in yeast extract peptone dextrose (YPD) liquid medium (Land Bridge Co. Ltd) and cultured in an oscillator at 30°C and 200 rpm. The growth of \textit{L. monocytogenes} and yeast NMY51 was spectrophotometrically measured with optical density (OD) at 600 nm (UV-Vis spectrophotometer, U-3900; Hitachi).

2.2 Effect of durancin GL on \textit{Listeria monocytogenes} growth curve

Durancin GL was prepared according to the method of Ju et al. (2015) Bacteriocin titer was determined according to the literature (Van Reenen, Dicks, & Chikindas, 1998), and the unit of bacteriocin titer is AU/ml. \textit{L. monocytogenes} Scott A was inoculated into BHI liquid medium with 1% (v/v) inoculation volume. A final concentration of durancin GL at 100 AU/ml or 200 AU/ml was added to the BHI culture when \textit{L. monocytogenes} was in logarithmic growth period. The growth of \textit{L. monocytogenes} was spectrophotometrically measured. According to the literature (Andrews, 2001), the live bacteria in 100 μl culture above were enumerated on BHI agar plate after serial dilution.

2.3 Effect of durancin GL on carbohydrate utilization by \textit{Listeria monocytogenes}

According to Premaratne, Lin, & Johnson, (1991), the basic medium with the lowest requirement for the growth of
L. monocytogenes was prepared, and the only carbohydrates used in the basic medium above were glucose, fructose, mannose, cellobiose, trehalose, and maltose, respectively. All the compounded carbohydrate medium was sterilized at 121°C for 15 min. After the medium was cooled to room temperature, a certain concentration of duracin GL was added in with a final concentration of 0 AU/ml (as control), 50 AU/ml, 100 AU/ml, and 200 AU/ml, respectively. Then, the growth of L. monocytogenes was followed by OD<sub>600</sub> value.

### 2.4 Gene knockout box construction

According to the manufacturer’s protocol of bacterial genome extraction kit (Sangon Biotech), the genomic DNA in L. monocytogenes was extracted using SZ-10 column silica gel membrane. Man-PTS IIC knockout box was constructed with primer pairs Pr01 (containing Sal I restriction site)/Pr02 and Pr03/Pr04 (containing Bgl II restriction site), and Man-PTS IID knockout box was constructed with primer pairs Pr05 (containing Nco I restriction site)/Pr06 and Pr07/Pr08.
mid DNA per mL

Referring to the optimized method by Park & Stewart, (1990), L. monocytogenes cells were treated by penicillin (the final concentration was 10 μg/mL, Sigma-Aldrich LLC). With a ratio of 20 μg plasmid DNA per mL L. monocytogenes Scott A (1 × 10^{10} CFU/mL), the recombinant vectors were transformed into L. monocytogenes by electroporation at a field strength of 10 kV/cm (pulse duration 5 ms). The cells were plated on BHI agar plates containing penicillin (the final concentration was 50 μg/mL) to screen for the transformants. Single colonies on the plates were selected for PCR verification and then sent to Sangon Biotech Co., Ltd for sequence verification. The Man-PTS IIC and IID gene mutant strains were designated L. monocytogenes GKC and L. monocytogenes GKD, respectively.

2.5 | Screening of Listeria monocytogenes gene mutants

The sensitivity to durancin GL of L. monocytogenes mutants was tested on BHI agar plates with reference to the literature (Seegal & Holden, 1945). The concentration of durancin GL in the test was 50 AU/mL, 100 AU/mL, and 200 AU/mL, respectively. The effect of durancin GL (200 AU/mL) on the growth of L. monocytogenes mutants and the effect on carbohydrate (glucose and mannose) utilization of L. monocytogenes mutants were tested as the same method stated above.

2.6 | Characterization of Listeria monocytogenes gene mutants

The gene of durancin GL (GenBank: HQ696461.1), Man-PTS IIC, and IID (GenBank: AF397145.1) was designated as GL, IIC, and IID, respectively. According to the target gene, primers with two Sfi I restriction sites were designed to amplify these target genes. The general PCR system is 50 μl, consisting of 25.0 μl of 2× Fast Pfu fly PCR supermix (TransGen, Beijing, China), 1.0 μl of template DNA, 1.0 μl of forward primer, 1.0 μl of reverse primer, and 22.0 μl of ddH2O. The primer pairs of target gene GL, IIC, and IID were Pr09/Pr11, Pr12/Pr14, and Pr15/Pr17 for bait vectors of pBT3-SUC and pBT3-STE, respectively. The primer pairs of target gene GL, IIC, and IID were Pr09/Pr10, Pr12/Pr13, and Pr15/Pr16 for prey vector pPR3-N, respectively. The PCR conditions were as follows: preheating at 98°C for 5 min, 35 cycles of denaturation at 98°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 10 s (gene GL) or 24 s (gene IIC) or 27 s (gene IID), and final extension at 72°C for 10 min.

The amplified fragments of GL, IIC, and IID were digested overnight by Sfi I enzyme at 50°C. Then, they were linked to the same digested vectors pBT3-SUC, pBT3-STE, and pPR3-N. Sfi I enzyme was provided by Thermo Fisher Scientific Co., Ltd. The recombinant vectors were transformed into E. coli Top10 competent cells. And the recombinant E. coli Top10 was selected on LB agar plate containing ampicillin (100 g/ml). Positive single colony on the plate was selected and verified by colony PCR and sequencing.

2.8 | Self-activation test, cytotoxicity test, and intermolecular interaction test

The recombinant plasmids prepared above were transformed into yeast reporter strain NMY51 chemically competent cells (Table 2). The yeast cells were plated on the YPAD (Land Bridge Co. Ltd) agar plate and cultured at 30°C for 4 days. Three colonies of 28 transformed strains (Table 2) were randomly selected from each YPAD plate and diluted with 1 ml sterile water, and then inoculated on SD-TL agar plate and SD-TLHA agar plate. SD-TLHA agar plate contained a certain concentration of 3AT (the final concentration was 0 mM, 5 mM, 10 mM, and 30 mM, respectively).

2.9 | Determination of β-galactosidase in transformants

β-galactosidase in transformants was determined by HTX β-galactosidase kit (Dualsystems Biotech). The brief steps were as follows: Firstly, the transformant strains were inoculated with 1% (v/v) inoculum in SD-TL liquid medium and cultured at 30°C with agitation at 250 rpm for 24 hr. Secondly, the supernatant was discarded after centrifugation at 2,500 × g for 5 min. Thirdly, in 96-well plates, 100 μl pyrolysate mixture (mixed by 995 μl one-step lysis and assay reagent and 5 μl dye stock solution) was added into each reaction and incubated for 90 min, and then, OD_{615} and at last OD_{546} for each well were determined by microplate reader (SpectraMax M2e, Molecular Devices). Each sample was analyzed in triplicate.

3 | RESULTS

3.1 | Effect of durancin GL on Listeria monocytogenes growth

Previous studies have indicated that durancin GL has a targeted inhibitory effect on L. monocytogenes (Du et al., 2012; Ju et al., 2015). This study further confirmed the effect of durancin GL on L. monocytogenes growth (Figure 1). During the logarithmic growth period
(about 4 hr, OD$_{600} \approx 0.721$), the presence of durancin GL suspended the growth of $L$. monocytogenes as the increase of OD$_{600}$ was delayed in the experiment group. The total bacteria (expressed as the logarithm of the total number of colonies) dropped from 8.21 (control group, 0 AU/ml) to 7.73 (experimental group, 100 AU/ml) and 6.96 (experimental group, 200 AU/ml) after treated for 4 hr. The results of bacteria count showed that colonies of $L$. monocytogenes decreased more obviously in experimental group compared with the control group (Figure 1). In high concentration of durancin GL (200 AU/ml, Figure 1b) treatment, the growth of $L$. monocytogenes cells was more restricted than low concentration group (100 AU/ml, Figure 1a). The number of $L$. monocytogenes was strictly controlled for a long time (at least 8 hr) in the durancin GL treatment group.

### 3.2 Effect of durancin GL on carbohydrate utilization by $Listeria$ monocytogenes

Figure 2 shows the effect of durancin GL on the utilization of carbohydrates by $L$. monocytogenes. Based on improved minimum requirement medium, six kinds of sugar were selected as the only source of carbon in this study. The results showed that the presence of durancin GL affected the absorption and utilization of glucose (Figure 2a) and mannose (Figure 2e) by $L$. monocytogenes. After 10 hr, in the compounded glucose medium, the OD$_{600}$ was 0.7500, 0.4825, 0.1700, and 0.0500 for each group (0, 50, 100, and 200 AU/ml). And after 10 hr, in the compounded mannose medium, the OD$_{600}$ was 0.7586, 0.3234, 0.1578, and 0.0196 for each group (0, 50, 100, and 200 AU/ml). However, the absorption and utilization of the other four sugars (Figure 2b, c, d, and f) by $L$. monocytogenes were not affected by durancin GL, regardless of the concentrations (from 0 AU/ml to 200 AU/ml). With the increase of bacteriocin concentration, the absorption and utilization of glucose (Figure 2a) or mannose (Figure 2e) by $L$. monocytogenes were more limited.

### 3.3 Preparation and characterization of $Listeria$ monocytogenes mutants

Genomic DNA of $L$. monocytogenes was extracted, and the result of agarose gel electrophoresis showed it was mainly concentrated in
23200 bp (Figure 3a). The results were consistent with previous experiments (Wu et al., 2017). After sequencing the knockout vectors, the results regarding to the Man-PTS IIC and Man-PTS IID knockout boxes (Figure 3b) showed that the corresponding sequences were consistent with the design.

On BHI agar plate containing 50 AU/ml duracin GL, *L. monocytogenes* mutants (*L. monocytogenes* GKC and *L. monocytogenes* GKD) showed visible growth (Figure 3c). Further research showed that *L. monocytogenes* mutants (GKC and GKD) were no longer sensitive to the duracin GL (from 50 AU/ml to 200 AU/ml, Figure 3d). This study further confirmed the effect of duracin GL on growth of *L. monocytogenes* and its mutants (Figure 3e). During the logarithmic growth period, *L. monocytogenes* GKC and *L. monocytogenes* GKD were not affected by the presence of duracin GL (the final concentration was 200 AU/ml).

Figure 3f shows the effect of duracin GL on the utilization of glucose (e) and mannose (f) by *L. monocytogenes* GKC and *L. monocytogenes* GKD. The results indicated that Man-PTS IIC or IID gene knockout resulted in little effect of duracin GL (200 AU/ml) on sugar absorption and utilization of *L. monocytogenes* mutants, indicating that Man-PTS subunit IIC or IID could be related for the sensitivity of *L. monocytogenes* to duracin GL.

### 3.4 Intermolecular interaction between duracin GL and Man-PTS subunit IIC or IID

To identify the interaction between duracin GL and Man-PTS subunit IIC or IID, we first constructed the yeast two-hybrid split-ubiquitin system to detect this interaction. For this purpose, the

![Figure 1](image1.png) **FIGURE 1** Effect of duracin GL on growth curve of *Listeria monocytogenes*. Solid line is colony plate counting results (represented by logarithmic values), and dotted line is OD<sub>600</sub>. The arrow indicates that a certain amount of bacteriocin duracin GL, the final concentration is 100 AU/ml (a) and 200 AU/ml (b), respectively, is added into the culture system at this time.

![Figure 2](image2.png) **FIGURE 2** Effect of duracin GL on carbohydrate utilization of *Listeria monocytogenes*. (a) Glucose, (b) fructose, (c) cellobiose, (d) trehalose, (e) mannose, (f) maltose. A certain duracin GL was added to the medium, in which the final concentration was 0, 50, 100, and 200 AU/ml, respectively.
plasmids listed in Table 2 were constructed, and a pair of plasmids was introduced into yeast reporter strain NMY51, which has two nutritional compensatory reporter genes (HIS3, ADE2) and a color indicator reporter gene (LacZ). Thus, the interaction between durancin GL and Man-PTS subunit IIC or IID can be determined on the growth of yeast cells on the SD-TL and SD-TLHA plates, as well as LacZ-dependent color change after incubation in the presence of LacZ substrates (Figure 4).

In order to ensure the accuracy and correctness of intermolecular interaction results, cytotoxicity test and self-activation test of yeast reporter strain NMY51 with recombinant plasmids were verified (Figure 4a, 4b, and 4c). Cytotoxicity test showed that all the six bait vectors constructed (pBT3-STE-GL, pBT3-SUC-GL, pBT3-STE-IIC, pBT3-SUC-IIC, pBT3-STE-IID, and pBT3-SUC-IID, in Table 2) can be expressed smoothly in yeast cells (Figure 4c), and LacZ reporter gene was activated with β-galactosidase activity was much higher than the negative control group (Figure 4c and e). However, there is little self-activation for target gene (gene GL, IIC, and IID) located in bait vector (Figure 4b, Figure 4b). As we all know, a certain concentration of 3-AT could eliminate some background growth on the selection plates (Vidalain, Boxem, Ge, Li, & Vidal, 2004), and thus, it was necessary to add a certain concentration of 3-aminotriazole (3-AT, from 0 mM to 30 mM) for inhibiting self-activation (Figure 4a and 4). The results showed that 5 mM 3-AT was sufficient to inhibit the self-activation above.

As the positive control, yeast reporter strain NMY51 with vector pNubG-Fe65 and pTSU2-APP grew well on SD-TL and SD-TLHA plates and showed strong β-galactosidase activity (OD₆15/OD₅46 = 1.103) because of the interaction of expressed cytosolic protein Fe65 (amyloid beta A4 precursor protein-binding family B member 1) and APP (amyloid A4 precursor protein), but yeast reporter strain NMY51 with vector pPR3-N and pTSU2-APP did not
FIGURE 4 Detection of durancin GL and Man-PTS IIC or IID interaction using yeast two-hybrid split-ubiquitin assay. (A) and (B) Self-activation test of plasmids. The concentration of 3-AT is 0 mM, 5 mM, 10 mM, and 30 mM, respectively. (C) Cytotoxicity test of plasmids. (D) Intermolecular interaction between durancin GL and Man-PTS IIC or IID. The concentration of 3-AT is 5 mM. (E) Quantification of β-gal activity in yeast cells containing various combinations of plasmids. The results are the average from triplicate assays. (a) is corresponding to (A), (b) is corresponding to (B), and so on. (e) is the positive control group (pNubG-Fe65 × pTSU2-APP) and negative control group (pPR3-N × pTSU2-APP).
grow on SD-TLHA plates and showed little β-galactosidase activity (OD_{655}/OD_{546} = 0.890), which was used as a negative control. The results showed that yeast reporter strain NMY51 with vector pPR3-N-GL and pBT3-SUC-IIC or pPR3-N-IIC and pBT3-STE-GL or pPR3-N-IIC and pBT3-SUC-GL grew well on the SD-TL and SD-TLHA plates and showed a higher β-galactosidase activity than negative control, which OD_{655}/OD_{546} was 1.059, 1.153, and 1.129, respectively; however, yeast reporter strain NMY51 with vector pPR3-N-GL and pBT3-STE-IID or pPR3-N-GL and pBT3-SUC-IID did not grow on the SD-TLHA plates, and yeast reporter strain NMY51 with vector pPR3-N-IID and pBT3-STE-GL or pPR3-N-IID and pBT3-SUC-GL grew well same as the self-activation of pPR3-N-IID (Figure 4b). Based on the above experimental results, it has indicated that there was a strong interaction between durancin GL and Man-PTS subunit IIC. However, the interaction between durancin GL and Man-PTS subunit IID was not present or weak.

### Discussion

In order to clarify the targeting inhibition of *L. monocytogenes* by durancin GL, a new class Ila bacteriocin, *L. monocytogenes* mutants with Man-PTS IIC or IID deletion were constructed; then, its utilization of glucose and mannose and the sensitivity to durancin GL were investigated, and in further, the interactions between durancin GL and the subunits of IIC or IID in Man-PTS of *L. monocytogenes* were characterized by yeast two-hybrid split-ubiquitin system. The genetic and biochemical evidence provided in this study revealed that Man-PTS subunit IIC was the target/receptor for sensitivity of *L. monocytogenes* to durancin GL. Man-PTS subunit IID combined with durancin GL to form some kind of complex, which limited the carbohydrate utilization of glucose and mannose, thereby inhibiting the growth and reproduction of *L. monocytogenes*.

By monitoring the growth dynamics (Wu, Yin, Hsu, & Jiang, 2005), the inhibitory effect of pediocin ACCEL (class Ila, pediocin-like bacteriocins) on *L. monocytogenes* was similar to that in this study. Similar results of antilisterial bacteriocins have been reported in several studies involving bacteriocins of bactofencin A (O’Shea et al., 2013), plantaricin LPL-1 (Wang, Qin, Zhang, Wu, & Li, 2018), lactacin 3147 (Deegan, Cotter, Hill, & Ross, 2006), Apb 118 (Corr et al., 2007), and sakacin P (Tessema, Moretro, Kohler, Axelson, & Naterstad, 2009). Besides, the effect of mesentericin Y105, a class IIa bacteriocin, on four sugar utilization by sensitive bacteria showed that the sensitivity of *E. faecalis* was highly increased in the presence of glucose or mannose, compared to cellobiose or fructose (Hechard, Pelletier, Cenatiempo, & Frere, 2001), and the sensitivity of *L. monocytogenes* was affected in a medium supplemented with mannose or glucose but not with cellobiose or fructose (Karine, Yves, Pascale, & Yann, 2001). Many reports about Man-PTS components as bacteriocin receptors have been proved experimentally. It was reported that Lactococcin A affected on the carbohydrate utilization by *L. monocytogenes*, and the correlation between ptn gene and Lactococcin A sensitivity was verified by gene knockout and gene replacement experiments (Diep et al., 2007). It was an observation further supporting the notion that the Man-PTS components act as a receptor targeting for bacteriocin. To gain insight into bacteriocin resistance, four class Ila bacteriocin (pediocin PA-1) resistant mutants of *E. faecalis* were obtained, and further research data confirmed the important role of Man-PTS in class Ila bacteriocin sensitivity and we demonstrate its importance involving global carbon catabolite control (Opsata et al., 2010). Stoll and Goebel, (2010) examine the major PEP-dependent phosphotransferase systems of *L. monocytogenes* by a systematic deletion analysis and identified the major PTSs involved in glucose, mannose, and cellobiose transport. A growing number of reports suggest that Man-PTS components act as a receptor targeting for bacteriocin (Colombo et al., 2018). Besides, several researches revealed that the Man-PTS subunit IID also was target/receptor for the sensitivity of target bacteria on bacteriocin (Stoll & Goebel, 2010; Tymoszewska, Diep, & Aleksandrzak-Piekarczyk, 2018; Zhou et al., 2016). However, credible evidence was not observed in this study because of interaction between durancin GL and Man-PTS subunit IID was not present or weak.

Although several plasmids have been used for *L. monocytogenes* generating mutants by allelic exchange, construction of *L. monocytogenes* mutants has been inefficient due to lack of effective selection markers for first and second recombination events (Abdelhamed, Lawrence, & Karsi, 2015). A new suicide plasmid pHoss1 provides answers to the above questions. Gene knockout vector pHoss1 was constructed by Hossam et al. (Abdelhamed et al., 2015), which have successfully knocked out some genes of *L. monocytogenes*, and the success rate is 80%-100%. Based on the principle of homologous recombination, Man-PTS component IIC- and IID-related genes of *L. monocytogenes* were knocked out with the help of vector pHoss1, and the mutants of *L. monocytogenes* with deletions of Man-PTS IIC and IID were constructed in this study. Despite its great popularity, the greatest disadvantage of the classical yeast two-hybrid system is the obligatory nuclear localization of the proteins and, hence, their site of interaction. Five years after the initial description of the yeast two-hybrid system, an alternative with the potential to overcome these limitations was described. The split-ubiquitin system never managed to gain as much popularity as the yeast two-hybrid system despite providing the same ease of application, yet it allows virtually all protein types to be tested without the need to truncate or mislocalize these proteins and without introducing additional artifacts to those associated with the yeast two-hybrid system (Xing, Wallmeroth, Berendzen, & Grefen, 2016). The interaction between bacteriocin durancin GL and Man-PTS IIC or IID was detected by yeast two-hybrid split-ubiquitin system. Although both IIC and IID are components of Man-PTS, they interact with bacteriocins durancin GL in the opposite way. There are many possible reasons for this. Firstly, there is no interaction between bacteriocin durancin GL and Man-PTS IID. Secondly, because of the structural differences between Man-PTS IIC and Man-PTS IID, the former has 7 transmembrane regions, and the latter has four
transmembrane regions. Thirdly, it may also be related to the location of membrane subunit in yeast cells. All of the above may need further confirmation in future research.

Although this study has revealed the mechanism of bacteriocins durancin GL inhibiting L. monocytogenes, there are still a lot of unclear aspects in its physical and chemical properties, bacteriostasis process and related mechanism and so on. As a new bacteriocin, clarifying the relevant mechanism of durancin GL inhibiting L. monocytogenes is not only provides a new choice for the development of efficient natural food preservatives, but also provides a scientific basis for further clarifying the role of bacteriocins.

5 | CONCLUSION

In this study, results indicated that durancin GL, a new bacteriocin, had has obvious antibacterial activity against L. monocytogenes. The presence of durancin GL affected the absorption and utilization of glucose and mannose by L. monocytogenes; however, the L. monocytogenes Man-PTS gene mutants exposed to bacteriocin duracin GL still grow and utilize glucose and mannose in the medium. Furthermore, an obvious intermolecular interaction between durancin GL and Man-PTS subunit IIC is confirmed in this report. This provides a basis for sensitivity of L. monocytogenes to durancin GL.

ACKNOWLEDGMENTS
This project was financially supported by the National Natural Science Foundation of China (No 31271930) and the Innovation of Graduate Culturing of Jiangsu Province (KYLX_1167).

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

ETHICAL APPROVAL
The study did not involve any human or animal testing.

INFORMED CONSENT
Written informed consent was obtained from all study participants.

ORCID
Xueyou Wu https://orcid.org/0000-0002-5963-9883

REFERENCES
Abdelhamed, H., Lawrence, M. L., & Karsi, A. (2015). A novel suicide plasmid for efficient gene mutation in Listeria monocytogenes. Plasmid, 81, 1–8. https://doi.org/10.1016/j.plasmid.2015.05.003
Alvarez Ordonez, A., Leong, D., Hickey, B., Beaufort, A., & Jordan, K. (2015). The challenge of challenge testing to monitor Listeria monocytogenes growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document. Food Research International, 75, 233–243. https://doi.org/10.1016/j.foodres.2015.06.004
Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48(Suppl. 1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5
Bastos, M. D. C. D. F., Coelho, M. L. V., & Santos, O. C. D. S. (2015). Resistance to bacteriocins produced by Gram-positive bacteria. Microbiology, 161, 683–700. https://doi.org/10.1099/mic.0.88228-0
Colombo, N. S. R., Chalon, M. C., Navarro, S. A., & Bellomio, A. (2018). Pediocin-like bacteriocins: New perspectives on mechanism of action and immunity. Current Genetics, 64(2), 345–351. https://doi.org/10.1007/s00294-017-0757-9
Corr, S. C., Li, Y., Riedel, C. U., O’Toole, P. W., Hill, C., & Gahan, C. G. M. (2007). Bacteriocin production as a mechanism for the antif infective activity of Lactobacillus salivarius UCC118. Proceedings of the National Academy of Sciences of the United States of America, 104(18), 7617–7621. https://doi.org/10.1073/pnas.0700440104
Deegan, L. H., Cotter, P. D., Hill, C., & Ross, P. (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. International Dairy Journal, 16(9), 1058–1071. https://doi.org/10.1016/j.idairyj.2005.10.026
Diep, D. B., Skaugen, M., Salehian, Z., Holo, H., & Nes, I. F. (2007). Common mechanisms of target cell recognition and immunity for class II bacteriocins. Proceedings of the National Academy of Sciences of the United States of America, 104(7), 2384–2389. https://doi.org/10.1073/pnas.0608775104
Du, L., Liu, L., Liu, F., Ju, X., & Yuan, J. (2016). Phenotypic and genotypic alterations of durancin GL-resistant enterococcus durans strains. Foodborne Pathogens and Disease, 13(6), 325–332. https://doi.org/10.1089/fpd.2015.2094
Du, L., Somkuti, G. A., Renye, J. A. Jr, & Huo, G. (2012). Properties of Durancin GL, a new antilisterial bacteriocin produced by Enterococcus durans 41D. Journal of Food Safety, 32(1), 74–83. https://doi.org/10.1111/j.1745-4656.2011.00346.x
Favaro, L., Penna, A. L. B., & Todorov, S. D. (2015). Bacteriocinogenic LAB from cheeses - Application in biopreservation? Trends in Food Science & Technology, 41(1), 37–48. https://doi.org/10.1016/j.tifs.2014.09.001
Franz, C. M. A. P., den Besten, H. M. W., Böhnlein, C., Gareis, M., Zwietering, M. H., & Fusco, V. (2015). Microbial food safety in the 21st century: Emerging challenges and foodborne pathogenic bacteria. Trends in Food Science & Technology, 81, 155–158. https://doi.org/10.1016/j.tifs.2018.09.019
Gandhi, M., & Chikindas, M. L. (2007). Listeria: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology, 113(1), 1–15. https://doi.org/10.1016/j.ijfoodmicro.2006.07.008
Giaouris, E., Heir, E., Desvaux, M., Hebraud, M., Moretto, L., Langsrud, S., ... Simoes, M. (2015). Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Frontiers in Microbiology, 6, 841. https://doi.org/10.3389/fmicb.2015.00841
Gray, M. L., & Killinger, A. H. (1966). Listeria monocytogenes and listeric infections. Bacteriological Reviews, 30(2), 309–382.
Hechard, Y., Pelletier, C., Cenatiempo, Y., & Frere, J. (2001). Analysis of sigma(54)-dependent genes in Enterococcus faecalis: A mannos e PTS permease (EIIMan) is involved in sensitivity to a bacteriocin, mesentericin Y105. Microbiology, 147, 1575–1580.
Hechard, Y., & Sahil, H. G. (2002). Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. Biochimie, 84(5–6), 545–557. https://doi.org/10.1016/s0006-3345(02)01417-7
Ju, X., Chen, X., Du, L., Wu, X., Liu, F., & Yuan, J. (2015). Alanine-scanning mutational analysis of durancin GL reveals residues important for its antimicrobial activity. Journal of Agricultural and Food Chemistry, 63(28), 6402–6409. https://doi.org/10.1021/jacs.5b02114
Karine, D., Yves, C., Pascale, C., & Yann, H. (2001). A sigma(54)-dependent PTS permease of the mannose family is responsible for sensitivity of Listeria monocytogenes to mesentericin Y105. Microbiology, 147(Pt 12), 3263–3269. https://doi.org/10.1099/00221287-147-12-3263
Kjos, M., Nes, I. F., & Diep, D. B. (2009). Class II one-peptide bacteriocins target a phylogenetically defined subgroup of mannos e.
vivo. Plant Physiology, 171(2), 727–758. https://doi.org/10.1104/pp.16.00470

Zhou, W., Wang, G., Wang, C., Ren, F., & Hao, Y. (2016). Both IIC and IID components of mannose phosphotransferase system are involved in the specific recognition between immunity protein PedB and bacteriocin-receptor Complex. PLoS ONE, 11(10), e0164973. https://doi.org/10.1371/journal.pone.0164973

How to cite this article: Wu X, Ju X, Du L, Wang L, He R, Chen Z. The Man-PTS subunit IIC is responsible for the sensitivity of Listeria monocytogenes to duracin GL. Food Sci Nutr. 2020;8:150–161. https://doi.org/10.1002/fsn3.1285