The Time-interleaved Synthesis Mode of Plantaricin Regulated by Auto-inducing Peptide and Acetate in *Lactobacillus plantarum*

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ABSTRACT

The synthesis of plantaricin in *Lactobacillus plantarum* is regulated by quorum sensing system. However, the extracytoplasmic sensing domain of histidine kinase (PlnB1) and recognition of auto-inducing peptide remains unclear. We proved that key site of auto-inducing peptide PlnA1 is Ile-Ser-Met-Leu, which binds to the hydrophobic pocket Phe-Ala-Ser-Gln-Phe on loop 2 of PlnB1 by hydrophobic interactions and hydrogen bonding by site-directed mutagenesis of PlnA1. Moreover, a new inducer, acetate was found that regulate the plantaricin synthesis by binding to positive-charged pocket Arg-Arg-Tyr-Ser-His-Lys on loop 4 of PlnB1 via electrostatic interaction and van der Waals forces. The side chain of Phe143 on loop4 determined the specificity and affinity of PlnB1 to recognize acetate. In addition, the pheromone activity of PlnA1 and acetate are independent in vitro, but time-interleaved in vivo that PlnA1 activates quorum sensing in the logarithmic phase, whereas acetate in stable phase to maintain synthesis of plantaricin. The phenomenon that PlnA and

acetate time-interleaved regulate PlnB were proved versatile in the other three type of PlnB of *Lb. plantarum* in vitro and vivo. Finally, we propose a new model to explain the synthesis of plantaricin time-interleaved regulated by PlnA and acetate, which has potential applications in fermented food and pathogen prevention.

Keywords: plantaricin; pheromone activity; time-interleaved; histidine kinase; quorum sensing system.

INTRODUCTION

Quorum sensing is a process of cell-to-cell communication driven by diffusible auto-inducers, which regulate the behavior of bacteria, including virulence gene expression, biofilm formulation, swarming, antibiotic resistance and bacteriocin production[1,2]. Bacteriocins are proteinaceous compounds that inhibit microorganisms to provide a competitive advantage for the organisms that produce them or share immunity factors over a wide range of sensitive organisms that inhabit a common niche[3]. Bacteriocins fall into two classes: class-I lantibiotics that contain post-translationally modified lanthionine residues, and the class-II non-lantibiotics that do not exhibit extensive protein modification[4]. Plantaricin EF is a class-IIb two-peptide bacteriocin produced by *Lb. plantarum*, which exhibits strong activity against clinical and foodborne pathogens such as *Listeria*[5], *Staphylococcus*[6], *Salmonella*[7], *Escherichia*[8], *Campylobacter*[9], *Sheela*[10], methicillin-resistant *Staphylococcus aureus* (MRSA)[11] and vancomycin-resistant enterococci (VRE)[12].

The synthesis of plantaricin is regulated by quorum sensing based on the concentrations of autoinducer-2 (AI-2) and a specific auto-inducing peptide (plnA). AI-2 is a signaling molecule produced by a variety of Gram-positive bacteria[13] that co-regulates the synthesis of class II antimicrobial peptides from *Lactococcus lactis*, *Lb. plantarum* and *Lb. sakei* [14,15]. The mRNA levels of *plnEF* and corresponding antibacterial activity exhibit significant increases upon addition of artificial AI-2, and show decreases upon adding an AI-2 inhibitor (furanone)[16]. However, the sensor protein and the regulation mechanism of AI-2 in LAB remains unclear. Generally, the synthesis of plantaricin is regulated by the quorum sensing system encoded by *plnABCD* of *Lb. plantarum* WCFS1[17] and *plnNC8IF-HK-D* of *Lb. plantarum* NC8[18]. The *plnA* gene encodes the auto-inducing peptide (AIP) PlnA, and *plnB* the sensor histidine kinase (HK). The *plnC* and *plnD* genes encode the response regulators (RR) of Plantaricin synthesis[19]. PlnA has both antibacterial and pheromone activities, yet only the L-type of PlnA has the pheromone activity[20].

When the concentration of AIP (PlnA) is low, the histidine protein kinase (PlnB) is autophosphorylated before transfer of the phosphoryl group to the response regulator (PlnD) to activate the synthesis of plantaricin[21]. When the concentration of PlnA is high, then PlnB exhibits phosphatase activity to reduce the phosphorylation level of PlnD and inhibits plantaricin-related gene transcription[22,23].

Native PlnB1 has five transmembrane domains, which so far has proved refractory to purification and crystallization. Although, crystal structures of the

cytoplasmic domains of histidine kinases have been reported[24], few structures of the transmembrane and extracytoplasmic sensing (ES) domains of the kinases are available[25]. ES domains are more variable than cytoplasmic domains, consistent with their specialized roles in recognizing a variety of extracellular signals. The first identified ES domain was CitA of *Klebsiela pneumoniae*[26], which demonstrated how the packing of helices was affected by the ligand (citrate) binding. Subsequently, the ES domains of DcuS, AgrC and Tar have been identified , which accomplish ligand recognition by either hydrophobic or electrostatic interactions within their ligand‐binding pockets[27-30]. Variation in the nature of ES domain recognition is exemplified by PhoQ of *S. typhimurium* that binds to the ligand via membrane-exposed acidic surface in place of a ligand-binding pocket[26]. Although the plnA mediated synthesis of plantaricin has been established, the interaction of the AIP-HK requires further analysis[31]. Moreover, how the bacteriocin production is enhanced when upon co-culture with other LAB require explanation[32].

Here, we describe bioinformatics directed mutation of the plantaricin-related quorum sensing system of *Lb. plantarum* to identify key sites for PlnA1 binding to PlnB1 and locate the ES domain of PlnB1. Surprisingly, we found second site activation of PlnB1 by acetate and demonstrated versatile time-interleaved regulated the synthesis of plantaricin.

RESULTS

The plantaricin-related quorum sensing system of *Lb. plantarum* was divided into four types.

Previously, we isolated the *Lb. plantarum* 163 from traditional Chinese fermented vegetables, which showed a broad-spectrum antibacterial and antifungal activity[33,34]. In order to identify the regulation system of bacteriocin production of *Lb. plantarum*, 15 types of bacteriocin-related quorum sensing systems of *Lb. plantarum*, obtained from NBCI, were clustered and divided into four groups (Figure 1A), based on the composition of the quorum sensing system. PlnA was divided into four types, each of which had a conserved sequence Ser-Leu at the N-terminus (Figure 1B). The histidine kinase of *Lb. plantarum* 163, PlnB1, contained five transmembrane (TM) domains and four loops as shown in Figure.S1. The sequence of the loops was shown in Figure 1 C and D; loop 2 and loop 4 are in an extracytoplasmic location and might be the ES domain of PlnB1, which we sought to further characterize. Loop 2 had an α-helix and formed a hydrophobic pocket at the N-terminus (Figure S1) . Loop 4 was rich in positively charged amino acids and formed a positively charged pocket at the N-terminus (Figure S1). The hydrophobic pocket and the positively charged pocket might be the recognition sites for inducers. Furthermore, the quorum sensing of *Lb. plantarum* was divided into four types based on the composition, which were correlated to the classification of PlnA. Type 1, 2, and 4 of the quorum sensing system consisted of an inducer, a histidine kinase and a response regulator, while type 3 had two response regulators (Figure 1A).

PlnA and acetic acid (HAC) are inducers of the PlnABD quorum sensing system

We established a HPLC method to detect the activity of plnB (Figure S3). In this study, the sensor activity of plnB (ATPase) is the depletion of ATP (Figure S4); and the kinase activity of plnB was represented by the phosphorylation level of plnD (Figure S6). The quorum sensing system is regulated by AI-2 and the furanone is an inhibitor of AI-2. Synthesis of bacteriocin in *Lb. plantarum* is regulated by AI-2 and PlnA, the yield of bacteriocin decreases if furanone is added[16]. We measured the relationship between concentrations of AI-2 and pheromone activity (ATPase) of the supernatants of *Escherichia coli*, *Staphylococcus. aureus, Vibrio harveyi* and five lactic acid bacteria (Figure 2A 2B). Results showed ATPase activity of bacteria was not reduced after the addition of furanone, which indicated that AI-2 could not activate PlnB in vitro. However, the AI-2 did enhance the transcription of *plnEF* in vivo (Figure 2E, 2F). This suggested that AI-2 regulates the plantaricin synthesis via other pathways, such as LuxS, rather than via the plnABD pathway. Surprisingly, the pheromone activity was related to the concentration of HAC, yet other short-chain fatty acids had weaker pheromone activity (Figure 2C). These results indicated that HAC might be a special inducer to PlnB1.

In addition, four types of PlnA were capable of activating PlnB1 in *Lb. plantarum* to different degrees, but PlnA1 is the most efficient inducer, suggesting that the activity of PlnA is not strain-specific. Subsequently, we measured the optimal

inducible concentrations of the four types of PlnA and HAC (1 mg/L [plnA1 and A2], 10 mg/L [plnA3 and A4], and 1 g/L HAC) (Figure 2D). Finally, PlnA and HAC were used as inducers in *Lb. plantarum* 463(*plnA*$^{-}$*plnB*$^{+}$) to verify synthesis regulation of plantaricin. The mRNA levels of *plnE* and *plnF* increased to 30-folds after the addition of PlnA1 and HAC for 1 hour (Figure 2E). The yield of plantaricin E and F were increased to 160% and 350% compared to *Lb. plantarum* 163 and *Lb. plantarum* 463(*plnA*$^{-}$*plnB*$^{+}$), respectively (Figure 2F). These results are evidence that PlnA and HAC are important inducers of plantaricin synthesis by regulating PlnB and promoting PlnC or PlnD. However, how do PlnA and HAC recognize PlnB is still unclear. Some key amino acids of PlnA1 or group of HAC might play a role in the recognition of PlnB1.

Conserved sequences at the N-terminus are key sites of PlnA1.

Previously, we found that the pheromone activity of PlnA was less critical to activate PlnB1. In order to identify key sites of PlnA1, we changed the amino acid of PlnA1 to alanine. The pheromone activity of PlnA1 mutant I32A (55%), S33A (67%), and L34A (40%) decreased significantly (Figure 3A). To measure the interaction model of N-terminal conserved region, we changed the hydrophobic properties of Ile32, Ser33 and Leu34 (Figure 3B). Pheromone activity of PlnA1 decreased significantly when the hydrophobicity of Ile32 or Leu34 was reduced (I32N and L34N). Moreover, the hydrophilic nature of Ser33 is essential for its pheromone activity of PlnA1 (S33F). These results indicate that the key site of PlnA1 might be

169 located in the Ile31-Leu34 region. To confirm this, we synthesized short peptides that
170 were able to activate PlnB, in which the pheromone activity was 32% (Ser-Leu-Met),
171 66% (Ile-Ser-Leu-Met), 45%(Ser-Leu-Met-Tyr), and 68%(Ile-Ser-Leu-Met-Tyr)
172 compared to wild type PlnA1.(Figure 3C) Although changes in hydrophobicity and
173 charge at the middle and C-terminals did not have a significant effect on the activity
174 of PlnA1, the structural integrity is essential for PlnA1 to maintain its full pheromone
175 activity. To determine if PlnB1 could phosphorylate PlnD, we measured the
176 phosphorylation of PlnD in vitro to show that PlnA1 could promote the transfer of a
177 phosphoryl group to PlnD via PlnB1 activation. Phosphorylation levels of PlnD
178 showed a similar trend in the ATPase activity of PlnB1, which suggested that a plnA
179 mutant could affect the phosphorylation levels of PlnD by altering the ATPase activity
180 of PlnB1.

181 Further, pheromone activity of PlnA1 was detected in *Lb. plantarum*
182 463(*plnA*$^{-}$*plnB*$^{+}$) and both mRNA levels and yield of plantaricin from *plnE* and *plnF*
183 decreased significantly, when PlnA1 mutants were added compared to wild type
184 (Figure 3D 3F). The mRNA data and yield of plantaricin activated by PlnA1 mutants
185 is shown in Figure S4. The aforementioned results allows us to conclude that the key
186 sites of PlnA1 are Ile32, Ser33, and Leu34. Further, the
187 hydrophobic-hydrophilic-hydrophobic structure is essential to maintain the
188 pheromone activity of PlnA1 and the recognition of PlnB1.

189 **Recognition sites for PlnA1 and HAC on PlnB1 are different.**

In order to determine the binding domain of inducers, four loops of PlnB1 were individually deleted or deleted as a whole and expressed in *E. coli* C43 (DE3) , and then purified (Table S1,Figure S2). When loop 2 in PlnB1 was deleted, the ATPase activity of PlnB1 was reduced significantly if even when PlnA1 was added, whereas, ATPase activity was not affected when HAC was added, implying the loop 2 in PlnB1 could be a sensing domain bonded PlnA1. In addition, when loop 4 was knocked out, the ATPase activity was almost depleted, despite the addition of HAC, whereas, it was not influenced with the addition of PlnA1, leading us to the hypothesis that loop 4 could be a sensing domain that interacts with HAC. In contrast, the reduction of ATPase activity was not significant when both loop 1 and loop 3 were deleted, suggesting that these two loops are not involved with inducer recognition. There was almost no ATPase activity when all loops were knocked out together (Figure 4A). Therefore, PlnA1 and HAC have different binding sites on PlnB1 and the path of activation is independent. The entirety of the activity data of PlnB1 mutants activated by PlnA1 mutants and HAC is shown in Figure S7. Enzyme characterization of PlnA1 and PlnB1 mutants showed that the changes of Km were not significant, while the Vmax decreased significantly (Table S1). These results indicate that the activity of PlnB1 is dependent on the activation level of PlnA.

Further experimental results verified that changing the hydrophobicity of Phe66 and Phe70 and the hydrophilicity of Ser68 in loop 2 significantly reduced PlnB1 activity, which was activated by PlnA1. Moreover, changing the charge of Arg145,

211 Arg146, His149, and Lys150 in loop 4 significantly reduced the ATPase activity of
212 PlnB1 activated by HAC (Figure 4B). Further, we reversed the hydrophobicity of
213 Phe66 and Phe70 to become hydrophilic and the positive charge of Arg145-Lys150 to
214 negative charge. In doing so, we found PlnB activity to be markedly decreased
215 (Figure 4C). This demonstrated that the suitable hydrophilic lipophilic balance (HLB)
216 of Phe66, Ser68, and Phe70 and the positive charge of Arg145-Lys150 in PlnB1 are
217 essential in recognizing PlnA1 and HAC. Phosphorylation of PlnD decreased
218 significantly if Phe66, Ser68, Phe70 of loop 2, and Arg145-Lys150 of loop 4 were
219 changed (Figure 4B), which was similar to the ATPase in PlnB1 that was activated by
220 PlnA1 mutants or HAC. Surprisingly, we found that Phe143 in loop 4 was important
221 to PlnB1 activity (F143A) and was activated by HAC (Figure 4D), and F143Y and
222 F143L mutants in loop 4 appeared to have a lower affinity and specificity to HAC
223 (Figure 4E). This indicates that the side chain of Phe143 affects the specificity of
224 PlnB1 interaction with HAC.

225 Finally, the effects of PlnB1 mutants on mRNA and plantaricin yield in *Lb.*
226 *plantarum* 363 (*plnA*$^{+}$*plnB*$^{-}$) were detected. mRNA levels of *plnE* as well as the yield
227 of plantaricin E was reduced significantly, when loop 2 was deleted and the
228 hydrophobic environment of Phe66 and Phe70 was changed. The same results were
229 observed when the charge of Arg145, His149 and Lys150 were reversed. The in vitro
230 and in vivo results proved that the hydrophobic-hydrophilic-hydrophobic
231 (Phe-Ala-Ser-Gln-Phe) structure of the N-terminus of loop 2 and the positive charge

(Arg-Arg-Tye-Ser-His-Lys) of the N-terminus of loop 4 played the important roles in the regulation of kinase activity in PlnB1. We demonstrated that key domains of PlnB1 capable of binding PlnA1 are located in loop2, and those capable of binding HAC in order to regulate plantaricin production are located in loop 4.

Time-interleaving of PlnA1 and acetate regulated the quorum sensing system

In the work described herein, we found HAC interacts with the Arg-Arg-Tye-Ser-His-Lys domain in loop 4, thereby activating PlnB1. The basic amino acid played a key role in the interaction with HAC, where electrostatic interaction was the main force. Therefore, we speculated that the acetate might be the actual inducer binding to PlnB1. To verify this hypothesis, we tested ATPase activity of the acid radical of sodium acetate, sodium chloride, sodium nitrate, sodium sulfate, sodium phosphate and sodium carbonate, which showed that only acetate had a strong pheromone activity (Figure 5A). The in vivo experiment showed that the mRNA level of *plnE* increased to 90-folds compared to the mRNA level in *Lb. plantarum* 463(*plnA*$^{-}$*plnB*$^{+}$) after the addition of sodium acetate (Figure 5B). This result provides evidence that the acetic radical was another pheromone capable of promoting plantaricin synthesis, in addition to PlnA1. To explore PlnA1, acetate, and plantaricin production regulation, we measured the ATPase activity in the presence of different concentrations of PlnA1, acetate, as well their mixtures, as shown in Figure 5C 5D and Table S2. Experimental results demonstrated that the concentration of the plnA1

and sodium acetate, exhibited maximum kinase activity of PlnB1 and the maximum phosphorylation levels of plnD, were 1 mg/L and 1 g/L, respectively (Figure 5C). Moreover, we investigated the effect of adding plnA in combination with acetate on plnB activity and plnD phosphorylation. Comparing the results illustrated in Figure 5C and 5D, it was found that there was no inhibition or synergy action between PlnA1 and acetate, suggesting that PlnA and HAC act independently in the regulation of PlnB1 activity and phosphorylation of PlnD.

We next tried to determine how PlnA1 and acetate regulate quorum-sensing systems during the growth of *Lb. plantarum*. To accomplish this, we measured the related parameters of *Lb. plantarum* 163 as depicted in Figure 6A. The results showed that the concentration of PlnA1 was consistent with the trend of cell density and reaching a maximum (10 mg/L) at 24 hours. However, the optimal concentration of PlnA1 was 1 mg/L (Figure 2D). This showed that kinase activity of PlnB1, induced by PlnA1, was inhibited after 24 hours. Interestingly, the concentration of HAC increased rapidly after 24 hours, making up the lost kinase activity of PlnB1, which maintains the transcription of *plnE* and the synthesis of plantaricin E after 24 hours (Figure 6A). Therefore, we hypothesized that time-interleaving of PlnA1 and HAC would regulate synthesis of plantaricin synthesis. To test this, we examined the effect of the plnB1 mutants on plantaricin synthesis in vivo by adding the inducer, PlnA1, and HAC (Figure 6B). At the early stage (before 24 hours), the mRNA levels of *plnE* increased significantly when PlnA1 and HAC were added. Further, mRNA levels of *plnE* in *Lb.*

plantarum 663 (*plnA*$^{-}$*plnB*$_{loop4}^{-}$) increased significantly after adding PlnA1 at the late stage (after 24 hours) (Figure 6C 6D). This showed that pheromone activities of PlnA and HAC are independent and time- interleaved in *vivo*. Overall, PlnA1 and acetate are time-interleaved and regulate the plantaricin-related quorum sensing systems during the growth of *Lb. plantarum.* PlnA1 and acetate play major roles in the early stage and in the late stage, respectively.

Time-interleaved PlnA and acetate regulating the synthesis of plantaricin is universal in *Lb. plantarum*.

In order to assess whether the mode of regulation is specific to *Lb. plantarum* 163 or universal in *Lb. plantarum*, we cloned the other three types of PlnB (Figure 1A) and constructed their mutants by deleting loop 2 and loop 4 (Table S3). The results of PlnB2, PlnB3, PlnB4 and their mutants showed similar results to PlnB1. Activity of PlnB activated by PlnA or acetate decreased significantly if loop 2 or loop 4 was deleted (Figure 7A).

Subsequently, we transformed the four types of PlnB into *Lb. plantarum* 263 (*plnA*$^{-}$*plnB*$^{-}$), using the respective PlnA and HAC as inducers. In doing so, the mRNA levels of *plnE* and *plnF* were increased to 80-folds (Figure 7B). Moreover, the yield of plantaricin E and F returned to the level of wild type strains (Figure 7D). This indicated that the mode of PlnA and HAC undergo time-interleaved regulation, and the synthesis of plantaricin is universal in *Lb. plantarum*.

The predicted model of recognition between PlnB1, PlnA1 and HAC

The PlnA1 (Ile-Ser-Leu-Met) and acetate bind to hydrophobic and positively charged pockets by hydrophobic interactions, hydrogen bonding, and electrostatic interactions. To determine the spatial conformation of PlnB1 after bonding PlnA1 or acetate, we constructed a *de novo* structure of loop 2 and loop 4 of PlnB1 by protein homology/analogY recognition engine Version 2.0 (Phyre2), because there are no relevant crystal structures to refer to. Docking results showed that side chains of Phe66, Gln69 and Phe70 of loop 2 formed a hydrophobic pocket (Figure 8D, 8E and S1), which bound to PlnA1 by hydrophobic interactions (Ile32-Phe66, Met35-Phe66, and Leu34-Phe70) (Figure 8B). The Ser68 and Gln69 were located at the bottom of pocket, which bound to PlnA1 via hydrogen bonds (Ser33-Phe70, and Ser33-Gln69 as shown in Figure S8). In addition, the side chains of Phe143, Arg145, Arg146, His149 and Lys150 formed a positively charged pocket, which bound acetate by electrostatic interactions (Figure 8C). The side chain of Phe143 might affect the affinity and specificity of PlnB1 to acetate or maintain the structure of the pocket via hydrophobic or steric effects, which is described in the structure of BvgS[35].

DISCUSSION

Quorum sensing is a method of communication employed by bacteria to coordinate a response amongst a population[1] and has been associated with biofilms, virulence factors, and bacteriocin synthesis[2]. Bacteriocin production is regulated by

317 AI-2 and AIP in LAB [14,15,36]. However, our experimental results proved that AI-2
318 could not activate PlnB1 in vitro, but does promote the synthesis of plantaricin *in vivo*.
319 This indicates that the synthesis of plantaricin is not only regulated by the plnABD
320 system, but also by other quorum sensing systems[14]. AI-2 might regulate the synthesis
321 of bacteriocin by a pathway of AI-2/LuxS that is incomplete in *Lb. plantarum* based
322 on quorum sensing system in the KEGG database
323 (https://www.genome.jp/kegg-bin/show_pathway?map=ko02024&show_description=
324 show). Future experiments should seek to identify the homologue of LuxP, LuxU and
325 LuxO in future experiments in the genomic data of *Lb. plantarum*.

326 Generally, PlnA is considered a signaling molecule (ligand) of *Lb. plantarum,*
327 which activates the sensor, PlnB[37]. PlnA and PlnB were divided into four types
328 according to its sequence. The four types of PlnA, which we now know are not strain
329 specific, enhanced the synthesis of plantaricin. The N-terminal conserved sequence
330 (Ser-Leu) of PlnA is the key site to bind PlnB1; and the
331 hydrophobic-hydrophilic-hydrophobic structure (Ile-Ser-Leu) is essential for its
332 pheromone activity (Figure 3). The recognition region of plnA on plnB also has a
333 similar structure (Phe-Ala-Ser-Gln-Phe-Ile). PlnA1 and PlnB1 may be recognized by
334 hydrophobic interactions and hydrogen bonding (Figure 8). The ligand-receptor
335 binding mode driven by hydrophobic interactions is common in quorum sensing
336 systems. The AIP binds to a putative hydrophobic pocket in the AgrC*,* which is
337 mediated by a highly conserved hydrophobic patch[38]. In addition, the competence

stimulating peptide (CSP), pentapeptide LamD558 and ComX interacted with sensor proteins via hydrophobic residues[1,39]. Although the key site Ile-Ser-Leu-Met could activate PlnB1, the integrity of the plnA structure is necessary for its pheromone activity. The complete PlnA can bind to the cell membrane through the electrostatic interactions with lecithin or glycoprotein[40]. This results increased the concentration of PlnA on the cell membrane, thereby enhancing the pheromone activity of PlnA.

Interestingly, we firstly discovered that acetate could activate plantaricin-related quorum sensing systems. The binding site of acetate on PlnB1 is located at a positively charged pocket (Arg-Arg-Tyr-Ser-His-Lys) which is recognized during electrostatic interactions. Electrostatic interactions are common in the recognition between ligand and sensor. Tar, NarX and CitA recognized nitrate, sulfate by basic amino acid arginine[41-43]. Furthermore, the hydrophobicity of the Phe143 side chains in this positively charged pocket plays an important role in maintaining pocket conformation and the specific recognition of acetate (Figure 4). Furthermore, the specificity of PlnB1 to acetate was weakened when the Phe143 was replaced by Leu or Tyr, which may be activated by propionic acid and lactic acid. A similar phenomenon was found in BvgS, which the phenylalanine side chain recognized acetate and maintained cavity conformation by hydrogen bonds and van der Waals forces[35]. Regulation of acetate on plantaricin synthesis explains the increase in bacteriocin production after co-culture of *Lb. plantarum* and other LAB[44]. Histidine kinase has kinase activity and phosphatase activity depending on the concentration of

359 the inducer[45,46]. We found that PlnB1 exhibits kinase activity (PlnA1 < 1 mg/L,
360 sodium acetate < 5 g/L) and phosphatase activity (PlnA1 > 10 mg/L, sodium acetate >
361 10 g/L) (Figure 5). However, PlnB1 could maintain kinase activity as long as either
362 PlnA1 or acetate is at a suitable concentration. Further, we showed that
363 time-interleaving of PlnA1 and acetate regulate plantaricin synthesis is universal in
364 other types of plnB from different strains including *Lb. plantarum* AS-8, TMW1.25,
365 WCFS1 and ST-III (Figure 7). Therefore, we can manipulate the synthesis of
366 plantaricin by adjusting the concentration of acetate in the environment.

367 Based on these observations, we proposed a universal model for the regulation of
368 a plantaricin-related quorum system (Figure 8A). The concentration of PlnA and
369 acetate accumulated with the growth of bacteria. In the logarithmic stage, the
370 concentration of acetate was too low to activate PlnB, yet PlnA was a major factor in
371 pheromone activity. At the stable stage, the PlnA concentration is high and inhibit the
372 kinase activity of PlnB, but acetate activates PlnB through another pathway to
373 maintain the synthesis of plantaricin. After autophosphorylation of PlnB on histidine
374 residues, the phosphoryl group is transferred to the aspartate of PlnD like other
375 histidine kinases[25]. Phosphorylated PlnD recognizes a specific base sequence of the
376 promoter and initiates the transcription of the plantaricin gene[47]. Unlike *Lb.*
377 *plantarum* 163 (Type 1), there are two highly homologous response regulators in *Lb.*
378 *plantarum* WCFS1 (Type 3), which could promote or inhibit transcription of *plnEF* or

379 *plnJK* [21]. Time-interleaved plnA and acetate t regulate the synthesis of plantaricin *in*
380 *vivo*.

381 Interestingly, *Lb. plantarum* 163 is a heterolactic fermentation strain that
382 produces HAC by using lactic acid to obtain energy when the pH is low or the
383 nutrients are deficient. This type of metabolism complements the regulation of
384 bacteriocins, which might be the result of mutual evolution[48,49]. According to this
385 model, we can increase the yield of plantaricin by controlling the concentration of
386 sodium acetate in the medium. Moreover, we can inhibit the growth of harmful
387 bacteria in fermented fruits, vegetables, meat, fish and silage by adding vinegar to
388 activate the plantaricin-related quorum sensing system of *Lb. plantarum*.

389

390 **METHODS**

391 **Microbial strain and medium**

392 *Lb. plantarum* 163, *Lb. acidophilus* NX2-6, *Lactococcus lactis* NZ3900, *Lb.*
393 *paracasei*, *Pediococcus lactis* were cultured in MRS medium at 33 ℃. *E. coli* and *S.*
394 *aureus* were cultured in LB medium at 37 ℃. *Vibrio harveyi* strains BB170 (AI-1
395 sensor$^-$, AI-2 sensor$^+$) and BB120 (AI-1 sensor$^+$, AI-2 sensor$^+$) were cultivated in
396 modified auto-inducer bioassay (AB) medium[50]. *E. coli* DH5α was used to sub-clone
397 vectors while *E. coli* BL21 (DE3) was used to express PlnD. *E. coli* C43 (DE3) was
398 used to expressed PlnB and its mutants. The vectors used in this study are shown
399 (Table 1). *E. coli* DH5α, *E. coli* BL21 (DE3) and *E. coli* C43 (DE3) were purchased

400 from Zoman (Beijing, China). *Bam*HI, *Xho*I and T4 DNA ligase were purchased from
401 Thermo fisher (Waltham, USA). Primers and peptides were synthesized via Genscript
402 (Nanjing, China). AI-2 was purchased from Glixx Laboratories Inc (Hopkinton, USA).
403 Acetic acid (HAC) and sodium acetate were purchased from Sigma-Aldrich China
404 (Shanghai, China).

405 Table 1 the expression vectors of PlnB and its mutants.

Vectors	Description	Reference
pET30a(+)	Expression vector, Kan^r, T_7 promoter, lac operator.	Novagen
pET30a-PlnB1	Sensor gene *PlnB* from *Lb. plantarum* 163	This study
pET30a-PlnB2	Sensor gene *PlnB* from *Lb. plantarum* CGMCC1.557	This study
pET30a-PlnB3	Sensor gene *PlnB* from *Lb. plantarum* WCFS1	This study
pET30a-PlnB4	Sensor gene *PlnB* from *Lb. plantarum* TMW1.25	This study
pET30a-PlnD	Transcriptional regulator gene *PlnD* from *Lb. plantarum* 163	This study
pET30a-PlnB1$_{loop1}^-$	Deletion loop1(N35-S38) of PlnB1	This study
pET30a-PlnB1$_{loop2}^-$	Deletion loop2(A67-F70) of PlnB1	This study
pET30a-PlnB1$_{loop3}^-$	Deletion loop3(V112-L115) of PlnB1	This study
pET30a-PlnB$_{1loop4}^-$	Deletion loop4(R145-H150) of PlnB1	This study
pET30a-PlnB2$_{loop2}^-$	Deletion loop2(A67-F70) of PlnB2	This study
pET30a-PlnB2$_{loop4}^-$	Deletion loop4(R145-H150) of PlnB2	This study
pET30a-PlnB3$_{loop2}^-$	Deletion loop2(I67-K70) of PlnB3	This study
pET30a-PlnB3$_{loop4}^-$	Deletion loop4(R148-G152) of PlnB3	This study
pET30a-PlnB4$_{loop2}^-$	Deletion loop2(V67-I70) of PlnB4	This study
pET30a-PlnB4$_{loop4}^-$	Deletion loop4(K145-S149) of PlnB4	This study
pET30a-PlnB1-F66A	PlnB1 mutant, the 66th amino acid (phenylalanine) was replaced by glycine.	This study
pET30a-PlnB1-mutants	Single amino acid mutation ofPlnB1 as above	This study
Strains		
Lb. plantarum 163	Wild strain, *plnA*$^+$*plnB*$^+$	Hu[33]
Lb. plantarum 263	Mutant of *Lb. plantarum* 163, *plnA*$^-$*plnB*$^-$	This study
Lb. plantarum 363 (*plnA*$^+$*plnB*$^-$)	Mutant of *Lb. plantarum* 163, *plnA*$^+$*plnB*$^-$	This study
Lb. plantarum 463 (*plnA*$^-$*plnB*$^+$)	Mutant of *Lb. plantarum* 163, *plnA*$^-$*plnB*$^+$	This study
Lb. plantarum 563 (*plnA*$^-$*plnB*$_{loop2}^-$)	Mutant of *Lb. plantarum* 163, *plnA*$^-$*plnB*$_{loop2}^-$	This study
Lb. plantarum 663 (*plnA*$^-$*plnB*$_{loop4}^-$)	Mutant of *Lb. plantarum* 163, *plnA*$^-$*plnB*$_{loop4}^-$	This study

Bioinformatic analysis of the plantaricin-related quorum sensing systems of *Lb. plantarum*

20 genomes of *Lb. plantarum* (Accession number: PRJNA191579, PRJNA32969, PRJNA415899, PRJNA445630, PRJNA292463, PRJNA257680, PRJNA343197, PRJNA186807, PRJNA352480, PRJNA289547, PRJNA271910, PRJDB1927, PRJNA413560, PRJNA291681, PRJNA494615, PRJNA390680, PRJNA289547, PRJNA323381, PRJNA49145, PRJNA474785) were download from NCBI database. Plantaricin-related quorum sensing systems were aligned and the phylogenetic tree was made by MEGA 7.0 (Figure 1G). The sequence of PlnA and PlnB were analyzed by ClustalW. The transmembrane structure of PlnB1 was predicted by DAS-TMfilter (http://mendel.imp.ac.at/sat/DAS/DAS.html) and TMHMM Server (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

Expression and purification of PlnB and PlnD

The PlnB1 were amplified and mutants were constructed by overlap extension PCR[51]. Then they were ligated to pET30a (+) and transformed into *E. coli* C43 (DE3), purified by ultra-high speed centrifugation (100,000 xg, 60min), Ni-NTA affinity chromatography (GE, HisTrap FF Crude) (eluted by 100 mM imidazole), anion exchange chromatography (GE, HiTrap SP HP) (eluted by 250 mM sodium chloride), and molecular sieve separation (GE, Superdex 200 increase 5/150GL) according to the methods described by Ivan[52] and Hande[53]. PlnD was transformed into *E. coli* BL21 (DE3), purified by Ni-NTA affinity chromatography (eluted by 200 mM

427 imidazole) and refolded in dialysis bag (Tris-HCl buffer, 50mM, pH7.5, containing
428 10mM GST and 1mM GSSH) according the methods described in “The condensed
429 protocols from molecular cloning: a laboratory manual”[54].

430 Kinase activity (ATPase) of PlnB was determined by analyzing the concentration
431 of ATP as follows: PlnA 0.1 mg/L(or supernatant of *Lb. plantarum* 163 10μL); PlnB
432 10 mg/L; PlnD 10 mg/L; and ATP 450 μM in PBS buffer (pH 7.0, 50 mM) at 40 ℃
433 for 30 min. Next, the mixture was incubated at 100 ℃ for 5 min to inactivate the
434 enzyme (PlnB). ATP concentration was detected by HPLC (Dionex, California, USA)
435 in 5% acetonitrile and 95% PBS buffer(pH 7.0, 50 mM) at 259 nm and Aligent
436 Eclipse XDB-C18 (5 μm, 4.6×250 mm) column.

437

438 **Identification of the inducer in the supernatant**

439 Five kinds of lactic acid bacteria (*Lb. plantarum* 163, *Lb. acidophilus* NX2-6,
440 *Lactococcus lactis* NZ3900, *Lb. paracasei*, *Pediococcus lactis*) were culture in MRS
441 medium at 30 ℃ for 48 hours. *E.coli* ATCC 35218 and *S.aureus* ATCC29213 were
442 cultured in LB medium at 37 ℃ for 24 hours. The AI-2 concentration of supernatant
443 was detected by *Vibrio harveyi* BB170 as described by Kim[55]. The concentration of
444 HAC was detected by HPLC in phosphate buffer (pH2.5, 50mM) and methanol
445 (90:10) at 210nm and Aligent SB C-18 (5 μm, 4.6×250 mm) column. Then the
446 pheromone activity of supernatant was presented by ATPase of PlnB as above.
447 Furanone (10 mg/L) was adding to the supernatant to inhibit the activity of AI-2. The

pheromone activity of 0.5 g/L of short-chain fatty acids such as formic acid, HAC, propionic acid, butyric acid, lactic acid were measured as above. The pheromone activity of different concentrations of four type of PlnA and HAC was measured to determine the optimal concentration.

Subsequently, four types of PlnA and HAC were added as an inducer to *Lb. plantarum* 463 (*plnA*$^{-}$*plnB*$^{+}$) in MRS medium (without sodium acetate). The mRNA level was analyzed by qPCR[56]. The yield of plantaricin E and F was detected by HPLC in acetonitrile and water (10:90) at 227 nm and Aligent Eclipse XDB-C18 (5 μm, 4.6×250 mm).

Detection of key sites of PlnA1 by site-directed mutation.

In order to identify key sites of PlnA1, a series of PlnA1 mutants were designed by alanine scanning mutation as shown in Figure 3A. Then the hydrophobic and charge was changed by site-directed mutagenesis as shown in Figure 3B. Short peptide SLM, ISLM, SLMY, and ISLMY were designed according to result of alanine scanning mutation and site-directed mutagenesis. Four type of PlnA (Figure 1A) and mutants were synthesized via Genscript (Nanjing, China). The pheromone activity of PlnA and mutants were presented by ATPase of PlnB as above.

Detection of sensing domain and key sites of PlnB

Four kinds of PlnB mutants with loop removed (Table 1) were expressed and purified as above. Then the hydrophobic and charge were changed by alanine scanning mutation and site-directed mutagenesis as shown in Figure 4B 4C. The ATPase activity of PlnB activated by PlnA and HAC was detected as described above.

Detection of phosphorylation levels of PlnD

The phosphorylation level of PlnD were detected as follows; PlnA 0.1 mg/L (or HAC 0.5g/L), PlnB 10 mg/L, PlnD 100 mg/L and ATP 450 μM in PBS buffer (pH 7.0, 50 mM) at 40 ℃ for 30 min. Furthermore, the PlnA and HAC were added to the mixture to 100 mg/L and 5 g/L and incubated at 40 ℃ for 30 min. Then mixtures were separated via SDS-PAGE and transformed onto a nitrocellulose membrane. The phosphorylation level of PlnD was detected according to the protocol of Phosbind-biotin BTL-104kit (APExBIO, Houston, USA) (https://www.apexbt.com/life-science/protein-phosphorylation-research/phos-binding-reagent-biotin.html).

Analysis of the action mode of PlnA and acetate

The pheromone activity of acid radical of sodium acetate, sodium chloride, sodium nitrate, sodium sulfate, sodium phosphate and sodium carbonate were detected as above. Moreover, they were added (0.5g/L) to *Lb. plantarum* 463 ($plnA^{-}plnB^{+}$) as inducers and the mRNA levels of *plnE* was measures by qPCR.

The pheromone activity of mixtures containing different concentrations of PlnA1 (0-1000 mg/L) and different concentrations of sodium acetate (0-5g/L) were detected as above. The Km, Vmax and Kcat of PlnB1 and its mutants were measured as described by Jambovane and Eisenthal[57,58] Furthermore the cell density, pheromone activity, mRNA level of *plnE*, and yield of PlnA1, plantaricin E, HAC of *Lb. plantarum* 163 were detected at different hours (6, 12, 24, 36,and 48 hours) as above. Besides, the mRNA level of *plnE* of *Lb. plantarum* 163, *Lb. plantarum* 563 ($plnA^{-}plnB_{loop2}^{-}$) and *Lb. plantarum* 663 ($plnA^{-}plnB_{loop4}^{-}$) were detected by qPCR after adding PlnA1 or HAC at 5 and 35 hours.

Verification of the general mode of PlnA and acetate action

PlnB2, PlnB3 ,PlnB4 and their mutants were expressed as shown in Table 1. Then the pheromone activity of PlnA2, PlnA3, PlnA4 and HAC were detected as above. Finally, PlnB1, PlnB2, PlnB3 and PlnB4 were amplified and ligated to pMG36e, transformed into *Lb. plantarum* 263 ($plnA^{-}plnB^{-}$). Subsequently, they were induced by PlnA1, PlnA2, PlnA3 and PlnA4, respectively. The mRNA level of *plnEF* and the yield of plantaricin EF were detected as above.

Simulating the spatial model of the binding of PlnA and PlnB

The secondary structure of PlnA1 and PlnB1 was predicted by Prabi (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html) and Jpred 4

(http://www.compbio.dundee.ac.uk/jpred/). The hydrophobicity and charge were analyzed via ProtParam (https://web.expasy.org/protparam/) and DNAMAN (https://www.lynnon.com/). The spatial structure of PlnA1 and PlnB1 was predicted via the SwissModel (https://www.swissmodel.expasy.org/) and Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The reaction between PlnA1, acetate and PlnB1 were docked by ZDock Server (http://zdock.umassmed.edu/) and Autoduck (http://autodock.scripps.edu/).

LIST OF ABBREVIATIONS

Quorum sensing (QS)

lactic acid bacteria (LAB)

methicillin-resistant *Staphylococcus aureus* (MRSA)

vancomycin-resistant *enterococci* (VRE)

auto-inducing peptide (AIP)

auto-inducer (AI)

histidine kinase (HPK)

acetic acid (HAC)

transmembrane domain (TM)

Extracytoplasmic sensing domain (ES)

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

F.M. and Z.L. designed experiments. F.M.,xxxxxxxxxx performed experiments. F.M. and xxxxxxxx analyzed data. F.M. and xxxxxx wrote the paper. Xxxxxxxx modified the language.

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Supporting information

552 **S1 Fig**. schematic diagram of PlnB1 transmenbrane domains and loops.

553 The transmembrane structure of PlnB1 was predicted by DAS-TMfilter
554 (http://mendel.imp.ac.at/sat/DAS/DAS.html) and TMHMM Server
555 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). The amino acid was shown in one
556 letter, the yellow circle is a hydrophobic amino acid, the green circle is a hydrophilic
557 amino acid, the blue circle is a basic amino acid, and the red circle is an acidic amino
558 acid. The inducer plnA-SLMY and acetate were recognized by loop2 and loop4 of
559 plnB1 by hydrophobic interaction (yellow dotted line) and electrostatic interaction
560 (blue dotted line).

561 **S2 Fig**. Expression and purification of PlnB and plnD

562 **A** is the expression and purification of PlnB. Lane 1-5 are control group, crude
563 homogenate, purified via Ni-NTA affinity, purified by anion exchange
564 chromatography, and purified by molecular sieve separation, respectively. **B** is the
565 expression, purification, and refolding of PlnD. Lane 1-4 are control group, crude
566 homogenate, purified via Ni-NTA affinity, and refolding, respectively. **C** is expression
567 and purification of four type of plnB and its mutants. D is the expression and
568 purification of plnB1 mutants.

569 **S3 Fig.** standard curve of plantaricin, HAC, and ATP detected by HPLC

570 **A** is the HPLC result of ATP concentration incubated by the mixture (containing
571 plnA, plnB, plnD and ATP) at different from 0 to 60 min. The peak area of ATP is
572 decreased with time. **B** is the HPLC result of ATP concentration incubated by different

combinations of plnA, plnB and plnD. **C** is the standard curve of plantaricin yield measured by HPLC (mobile phase: 90% acetonitrile and 10% water, wavelength 227nm, Aligent Eclipse XDB-C18 column). **D** is the standard curve of HAC concentration measured by HPLC (mobile phase: 90% PBS pH2.5 and 10% methanol, wavelength 210nm, Aligent SB-C18 column). **E** is the standard curve of ATP measured by HPLC (mobile phase: 95% PBS and 5% acetonitrile, wavelength 259nm, Aligent Eclipse XDB-C18 column).

S4 Fig Effect of MRS components and pH on the ATPase of plnB. **A** is the ATPase activity of plnB induced by components of MRS medium. The sodium acetate could activate the ATPase activity of plnB. **B** is the effect of pH on the ATPase of PlnB1. it has highest activity at pH7.0.

S5. Fig. Effects of plnA and plnB mutants on phosphorylation of plnD

A shows the phosphorylation level of plnD are different while incubated by plnA mutants. **B** is the phosphorylation level of plnD incubated by plnB mutants and plnA1. It indicated that the plnD was refolded into activate form, and the plnB could recognize plnA (inducer signal) and phosphorylated plnD (histidine kinase).

S6. Fig. the mRNA levels and yield plantaricin of *Lb. plantarum* 463 ($plnA^{-}plnB^{+}$) are different after adding PlnA mutants for 1 hour. **A** is the mRNA level of *plnE* and *plnF.* **B** is the yield of plantaricin E and F of *Lb. plantarum* 463 ($plnA^{-}plnB^{+}$).

S7 Fig. Heat map of plnB mutants activated by plnA mutants.

The ATPase of plnB mutants activated by plnA mutants is described in different color as staff gauge shown.

S8 Fig. Docking models of PlnA1 and loop2 of PlnB1.

A is the docking model of PlnA1 and PlnB1. **B** is the enlarged view of key sites. It has four van der Waals force (N39-F66, V40-F66, S33-A67, and F29-I65) and four hydrogen bonding (S33-F70, and S33-Q69). C and D are the surface of hydrophobic and positive charge pockets.

T1 Tab. Enzyme characterizes of plnB mutants

T2 Tab. The pheromone activity of mixtures of plnA and HAC

T3 Tab. Details of PlnB loops

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Figure captions

Figure 1. Bioinformatics analysis of plantaricin-related quorum sensing system

A is phylogenetic tree of plantaricin-related quorum sensing system of *Lb. plantarum*; and schematic of *pln* locus of four type of *Lb. plantarum.* The quorum system (*plnABD* or *plnABCD*) was divided into four types and shown in red pink arrow. The plantaricin related genes (*plnEFI*) were shown in yellow arrow. **B** is the amino acid sequence of four type of PlnA produced by different *Lactobacillus plantarum* based on the classification of **A**. **C and D** are sequence of extracellular loops of PlnB of four type of *Lb. plantarum*.

Figure 2. plnA and acetic acid activate plnB1 in vitro.

A is the pheromone activity of supernatant of *E. coli*, *S. aureus*, and four kinds of lactic acid bacteria with and without furanone. **B** is the relative concentration of AI-2, the positive control is the supernatant of *Vibrio harveyi* BB170; and the concentration of acetic acid. **C** is the activation effect of different kinds of short-chain fatty acids on

818 plnB1. **D** is the activation effect of different concentrations of sodium acetate and four
819 type of PlnA on plnB1. **E** is mRNA levels of *plnE* and *plnF* of *Lb. plantarum* 263
820 induced by sodium acetate and four type of PlnA. **F** is the yield of plantaricin E and F
821 produced by *Lb. plantarum* 263 ($plnA^{-}plnB^{-}$) induced by sodium acetate and four type
822 of PlnA.
823
824 **Figure 3** The hydrophobic sequence at the N-terminus determines thepheromone
825 activity of plnA1
826 **A** is effect of alanine scanning mutation of PlnA to the pheromone activity. **B** is
827 the pheromone activity of PlnA mutants that changed hydrophobic (N-terminus) and
828 charge (C-terminus); and the phosphorylation level of plnD incubated by plnB1 and
829 plnA1 mutants. **C** is the activation effect of hydrophobic sequences at the N-terminus
830 of PlnA1 on plnB1 and the phosphorylation level of plnD. **D** and **E** are mRNA levels
831 and yield of plantaricin of *Lb. plantarum* 463 ($plnA^{-}plnB^{+}$) after adding plnA1 and
832 mutants. The wild strain was *Lb. plantarum* 163 ($plnA^{+}plnB^{+}$).
833
834 **Figure 4** plnA and acetic acid have different receptor regions of plnB1
835 **A** A is the effect on the activity of plnB1 after deleting the loop. **B** is the effect of
836 alanine scanning mutation on the activity of plnB1, and the phosphorylation level of
837 plnD incubated by plnB1 mutants. **C** is the effect of hydrophobic and charge on the
838 activity of PlnB1 mutants. **D** is effect of F143 mutation (F143L and F143Y) on the

specificity of plnB1 recognition of short-chain fatty acids. **F** is F143 mutation (F143L and F143Y) changes the binding ability of plnB1 to short-chain fatty acids. **F** and **G** are effects of plnB1 mutant on plantaricin synthesis (the mRNA levels and yield of plantaricin) of *Lb. plantarum* 363 ($plnA^{+}plnB^{-}$) and adding the synthetic plnA1 and acetic acid.

Figure 5 effects of plnA and acetate are independent, but depend on their concentration.

A is activation of different anions on plnB1. **B** is mRNA level of *plnE* of *Lb. plantarum* 463 ($plnA^{-}plnB^{+}$) after adding different anions for 1 hour. **C** shows the kinase and phosphatase of plnB1 was converted based on the concentration of inducer (plnA and acetate), and the phosphorylation level of plnD incubated by different concentration of PlnA1 and sodium acetate. **D** shows plnB1 maintains the activity of the kinase as long as one inducer (plnA or acetate) is at the appropriate concentration, and the phosphorylation level of plnD.

Figure 6 the synthesis of plantaricin regulated by PlnA and acetate is time-interleaving in *vivo.*

A is the cell density, yield of PlnA and HAC, mRNA level of *plnE*, yield of plantaricin E, and pheromone activity of supernatant during growth of *Lb. plantarum* 163. **B**, **C** and **D** are the mRNA levels in different host (*Lb. plantarum* 163 and its

mutants which sensor domain was deleted), and changes of mRNA after adding plnA and HAC at 5 and 35 hours.

Figure 7 PlnA and acetate time-interleaved regulate the PlnB is versatile in *Lb. plantarum*

A shows sensor domains are located at loop 2 (plnA) and loop4 (acetate) of 3 type plnB. **B and C** and **D** are the mRNA level and yield of plantaricin of *Lb. plantarum* 263 (*plnA*$^{-}$*plnB*$^{-}$) induced by PlnA and acetate when different PlnB transformed.

Figure 8 the model of PlnA and time-interleaved regulated PlnB in quorum sensing system and the docking result of recognition between plnB and inducer.

A is the schematic diagram of the regulation mechanism of plantaricin synthesis. **B** is the docking result of ISLM and loop2 of PlnB. **C** is the docking result if acetate and loop4 of PlnB. **D** and **E** are the docking result of recognition between inducer (ISLM and acetate) and loops (loop2 and loop4) of plnB that shown as surface.