

Supplementary materials

LuxR-Type Regulator RRP₆ Positively Regulates the Biosynthesis of Plantaricin EF and Improves Its Production in *Lactiplantibacillus* *plantarum* 163

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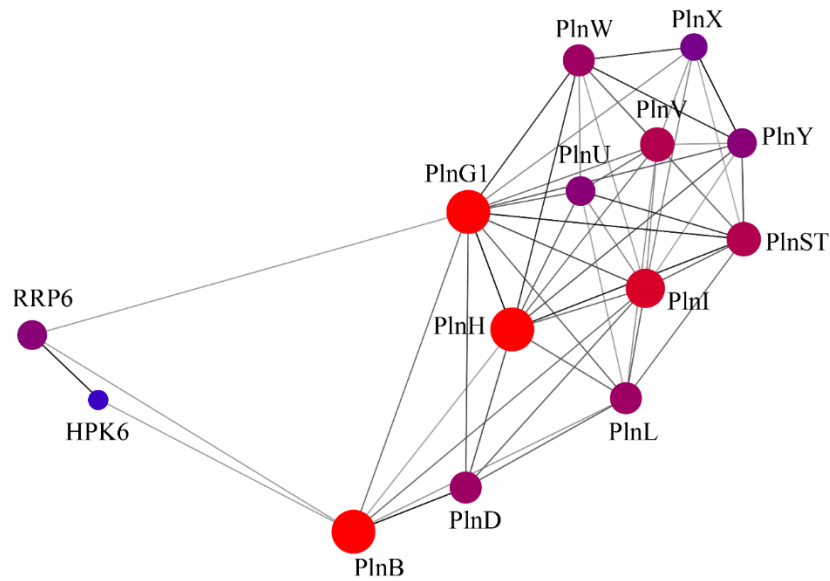


Figure S1 The genome, transcriptome and proteome data were analyzed using the BGI Cloud platform to identify the proteins that interact with the *pln* locus

Note: The color from purple to red indicates increasing interaction strength, and the thicker the line, the stronger interaction.

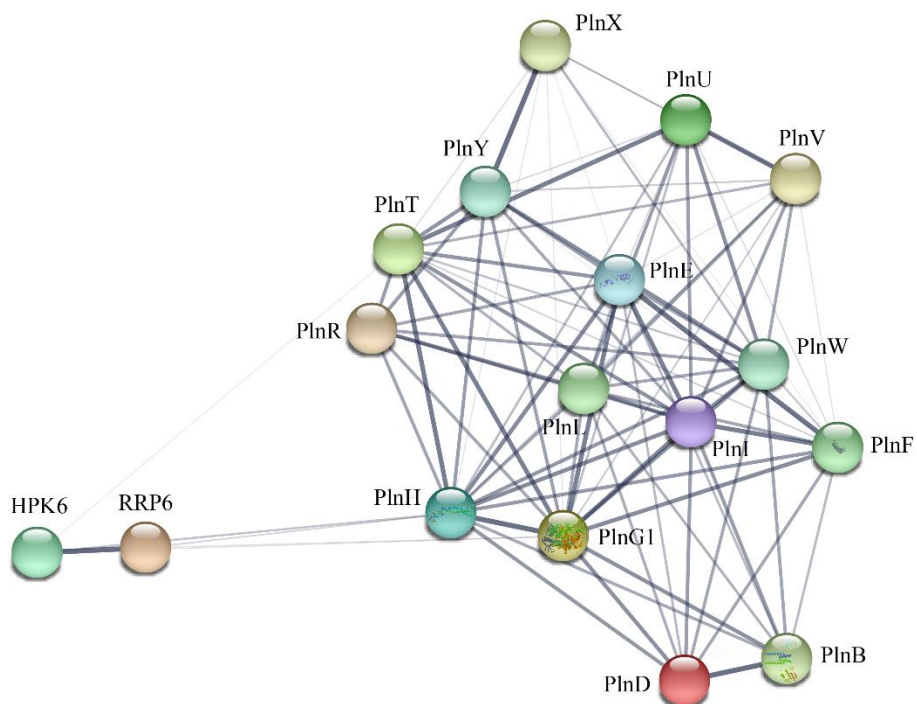


Figure S2 String network of protein-protein interaction to identify the proteins that interact with the *pln* locus

Note: The thicker the line indicates the stronger interaction.

Gene sequence of HPK₆ and RRP₆:

1. HPK₆ (1110 bp)

ATGGCGTTGTTGACGCATCATAAAAATAAATTGAAGACGATTGAATGGACGAGCTACGT
GTGGCTCGCCTATTTGCCGTATACAATTGCGGGTTACATACCCGTTAAATCTTGGCATGA
TTGGCTTTGGCTATTGCTTGTTGGTGGTTTTCTTGTTGTTGTACATCTTGTTGTGAGAA
GCCACATTGGCGTGACGTGACGATGCCAGCGGAGTTAGTTGTGACGGGACTGTTTGCG
GTATTTGCGGCTAACAATTTTATGATCATCTTTCCCGGCTGGCAAGTGAGCTTTTTACTT
GGTCGTCGTCCTAAACGAGAATTTTATGGTTTTGCTTTAGGGTATTATGCCTTTTTAATT
GGTGGCTGTATACGAGACTATTTACAATATCCTGGTGTGTTTGGGTGGCATAATGGTGAC
GTGATGGGCTTAGTTTTTCCATTGTTATCACCCATACTCGCGTATACCTTTTCGTGGTCA
GTTGAACGACAGCGCCAACTCAACCAAATAACCGCCGATTAAAAACAATTGTGGAA
CGTAACGAGCGTGAACGAATCGCGCGTGACTTGACGATACCTTGGACAAAGCTTTT
CAATGATTACACTCAAAACGGAATTAGCTAAAAAATTACTCGTCAAAGCACCGGAGAA
AGTGGCACCAGAATTGGACGAAATCGAACAAACCAGTCGCCAAAATTTACAGTTAGTT
CGCGAAATCGTCAATAATTTGCATGAACAATCGCTCACAGAAGTCCTATTGGCCCAAAC
TCGTAATCTAGCAGCGGTTGGTGTCTGGACGACGACCACTGGTGAGACACAGGCAAC
CAAGTGGCCGACAACGGTCCAAAGTTGTTTTGCGGCGGTGTTAGTTGAAGCCATGACC
AATGTGATGCGCCACGCACACGCGCACGAGGTGCGGATTGATTTTGTGAGACGGCAA
AAATTTATCAGATTAAGCTGAAAGATGACGGTAAAGGTGGAACCTTGATTCGTCAGGG
CGCCAACGGGATTGCCGGGATGCGAACCCGCTTGCAAGCTAAACAGGGCACCTTTGCC
ATCACGAGTAGTCGGCGCGGCACCCAGTTGATCTTAACCTTTACCAAAGGAGTAG(5'-3')

2. RRP₆ (606 bp)

ATGATAACTTTATACTTAGCAGAAGACCAAAGCATGTTGAATTCTGCCTTGACCCAATT
ACTGGAATTAGAAGATGATCTACACGTGGTGGGTAGCGCAGTAGACGGCATAAACGCC
TGGCAGGAGTTGCAAGAACTGCAACCGGATGTCGCAATTCTGGATATCGAAATGCCAG
GGATAACCGGCTTGATGTTGCTGATCTATTGGACAGTTCGCAGCTAGCCACGAAAGTA
ATAGTTTTGACGACTTTTGCGCAACGCCGTTATTTGAACGAGCAGTTAAAGCCAATGT
CGCGGGATATTTGTTAAAAGATAGTCCGAGTGATGACCTAATTGATGCAATTCGAGCTG
TGATGACCGGCCGCACCATCTATGCGCCCGAGCTAGTCACGAATATGTTGTCAGCAGAC
AACAATCCATTGACCGAACGGGAAGTAGCAGTTTTGGTCGAAGCTGAGAAGGGGTTG
CCCACGAAGACGATTGCGGCCAATTTATACTTGTGAGCGGGGACGACCCGTAATTACTT
ATCCGCCATTTTAGCAAATTGGGGGTTTACAATCGATTAGAAGCGATTCTGTGGCGA
AAGCTAATCAGTGGTTGTAA (5'-3')

Amino acid sequence of HPK₆:

1. HPK₆ (369 AA)

MALLTHHKNKLTIEWTSYVWLAYLPYTIAAYIPVKS WHDWLWLLLVGGFLVLYILVVEK
PHWRAVTMPAELVVTGLFAVFAANNFMIIFPGWQVSFLLGRRPKREFYGFALGYAFLIGG
CIRDYLQYPGVFGWHNGDVMGLVFLLSPILAYTFSWSVERQRQLNQTNRRRLKTIVERNE
RERIARDLHDTLGQSFSMITLKTTELAKKLLVKAPEKVAPELDEIEQTSRQNLQLVREIVNNL
HEQSLTEVLLAQTRNLAAGVWTTTTGETQATKWPTTVQSCFAAVLVEAMTNVMRHAH
AHEVRIDFVETAKIYQIKLKDDGKGGTLIRQGANGIAGMRTLQAKQGTFAITSSRRGTQL
ILTLPKE*

Amino acid sequence of RRP₆:

2. RRP₆ (201 AA)

MITLYLAEDQSMLNSALTQLELEDDLHVVGSAVDGINAWQELQELQPDVAILDIEMPGIT
GLDVADLLDSSQLATKIVIVLTTFQRRYFERAVKANVAGYLLKDSPSDDLIDAIRAVMTGR
TIYAPELVTNMLSADNNPLTERELAVLVEAEKGLPTKTIAANLYLSAGTTRNYLSAIFSKLG
VHNRLEAIRVAKANQWL*

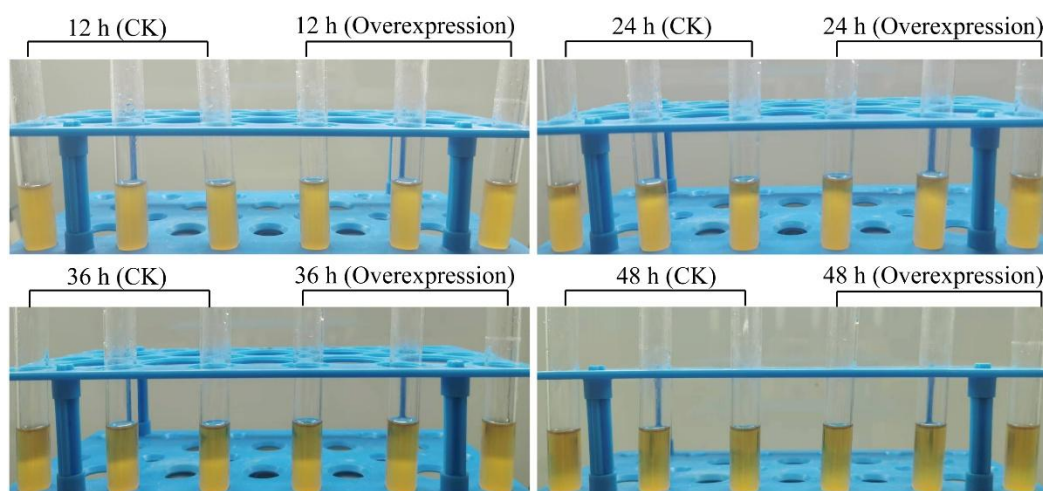


Figure S3 Effects of *rrp6* overexpression on the growth of *L. plantarum* 163

Note: CK: *L. plantarum* 163; Overexpression: *rrp6* gene overexpression in *L. plantarum* 163.

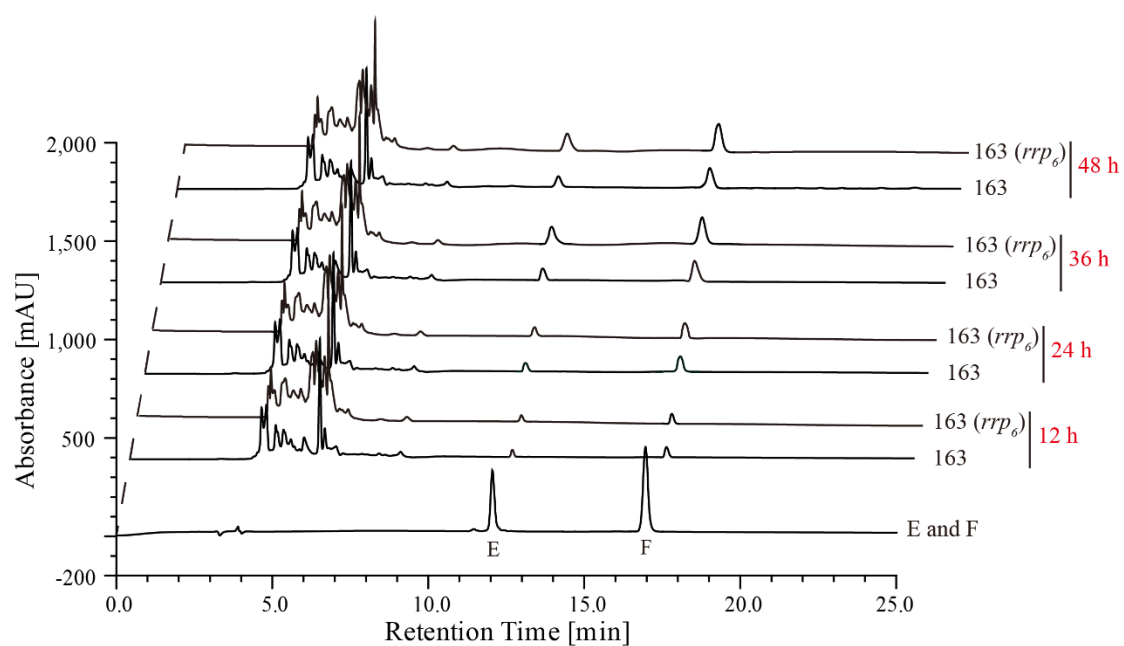


Figure S4 RP-HPLC analysis of plantaricin E and F produced by 163(*rrp6*)

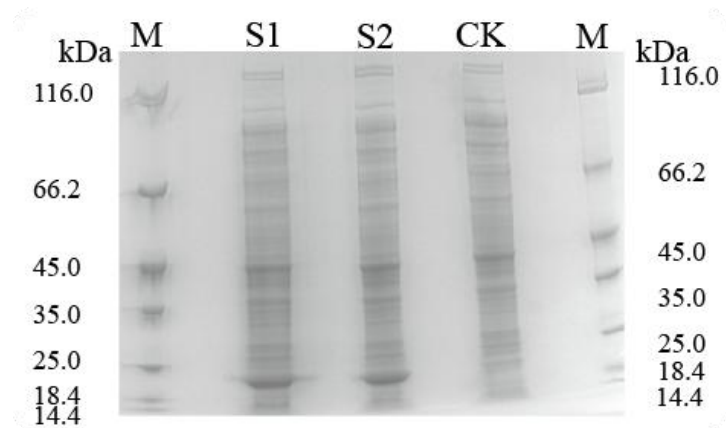


Figure S5 SDS-PAGE results of RRP₆ protein

Note: M: protein marker; CK: RRP₆ without induce; S1 and S2 are sample RRP6 after induced

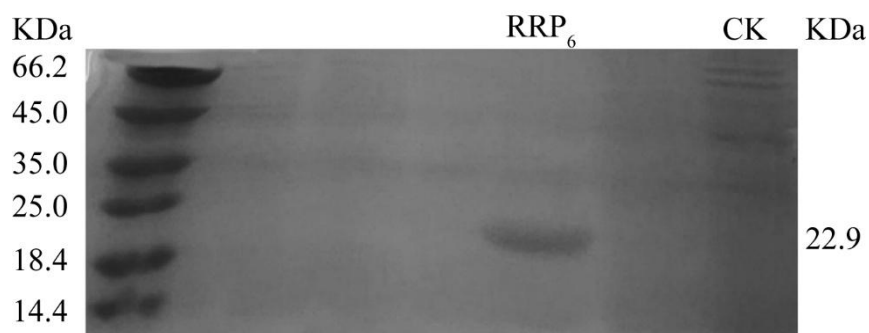


Figure S6 SDS-PAGE after RRP₆ purification

Note: M: protein marker; CK: RRP₆ without induce; RRP₆: purified protein of RRP₆.

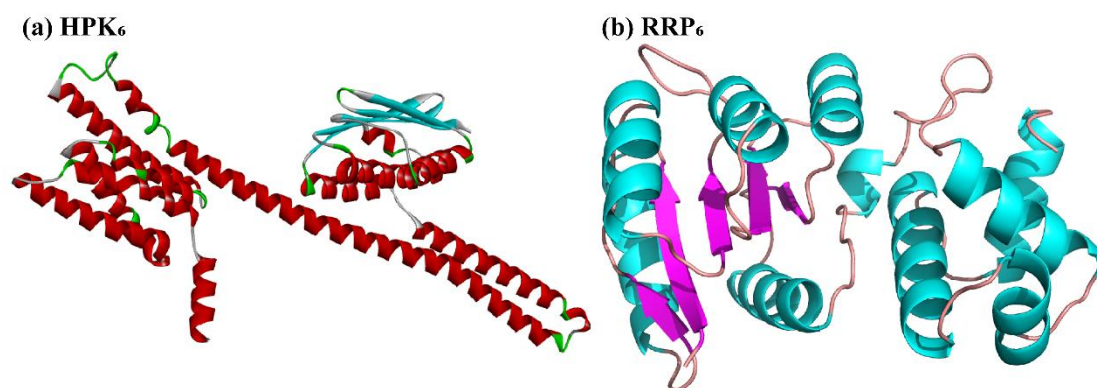


Figure S7 Homologous modeling of HPK₆ and RRP₆

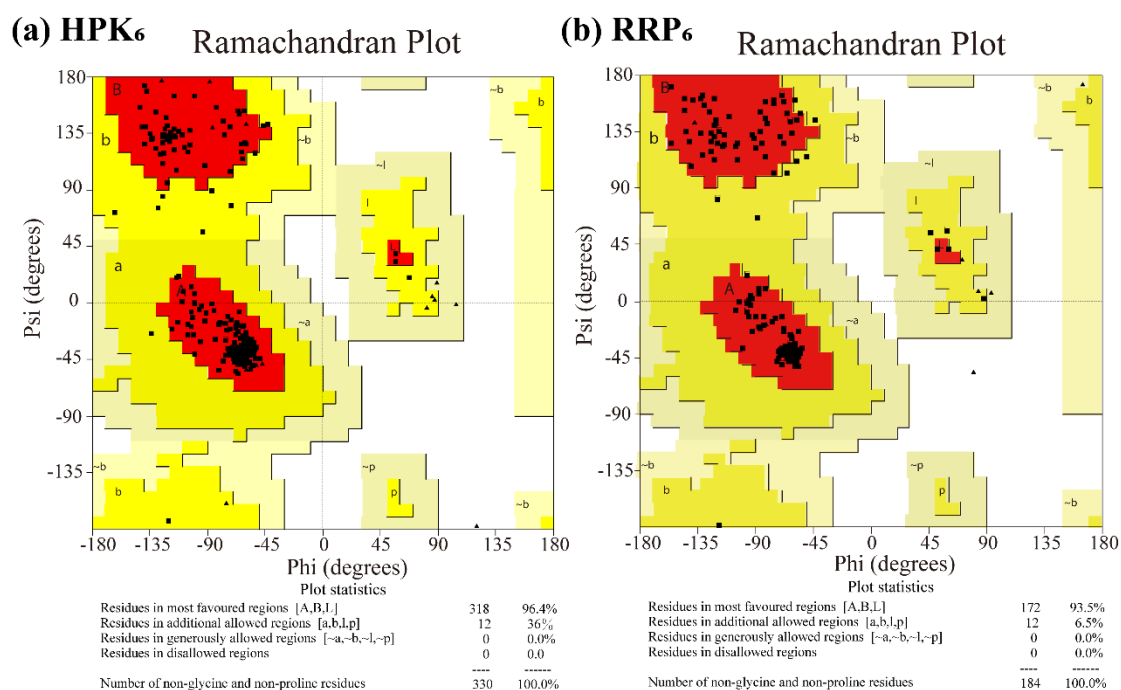
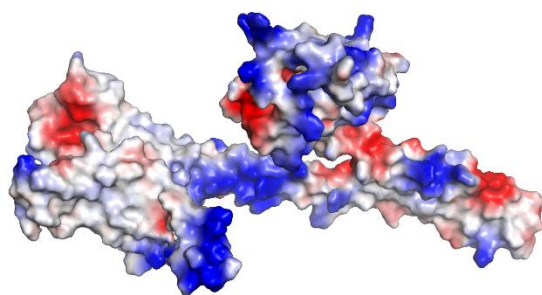


Figure S8 Ramchndran plot prediction of HPK₆ and RRP₆

(a) HPK₆



(b) RRP₆

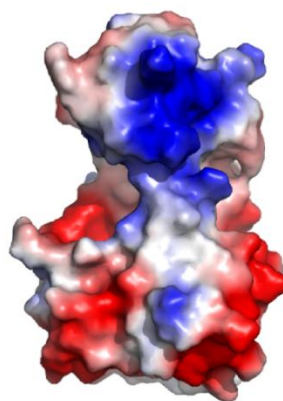
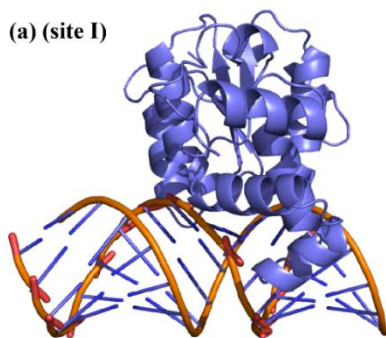


Figure S9 The static potential energy diagram of HPK₆ and RRP₆

(a) (site I)



(b) (site II)

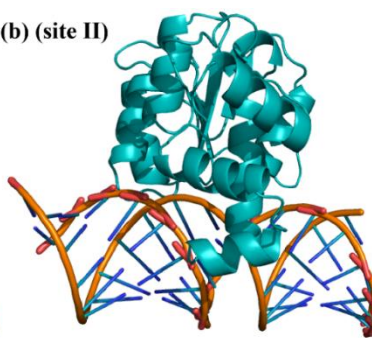


Figure S10 Molecular modeling of protein RRP₆ interaction with two binding sites.

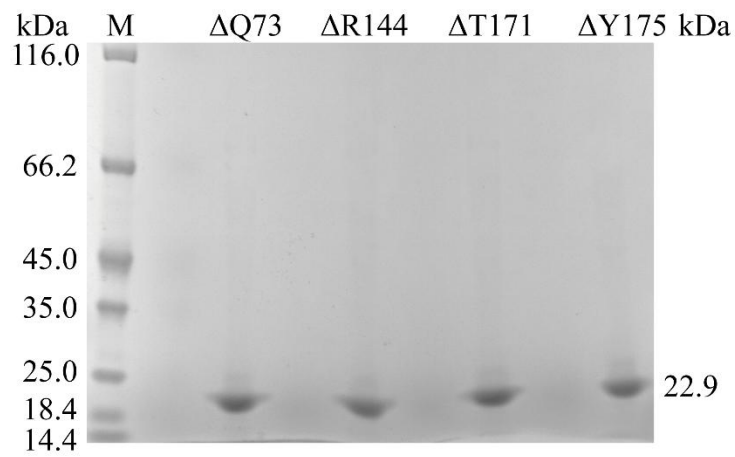


Figure S11 SDS-PAGE after mutant protein purification

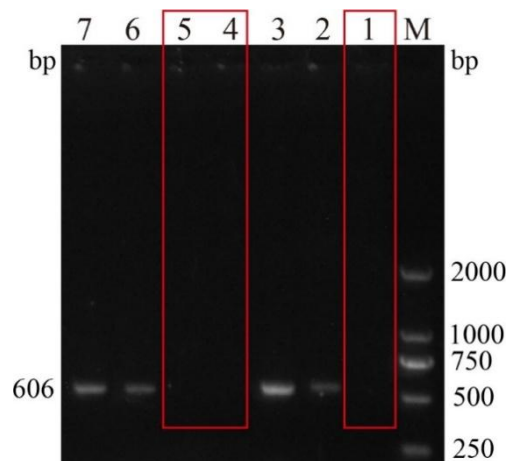


Figure S12 PCR verification of gene *rrp6* knockout strains

Note: M: DS2000 DNA marker; The size of the gene *rrp6* was 606 bp. PCR amplification was performed using the primers of the gene *rrp6*. The lanes (1, 4 and 5) where bands could not be amplified were the mutant strains of the gene *rrp6* knockout.

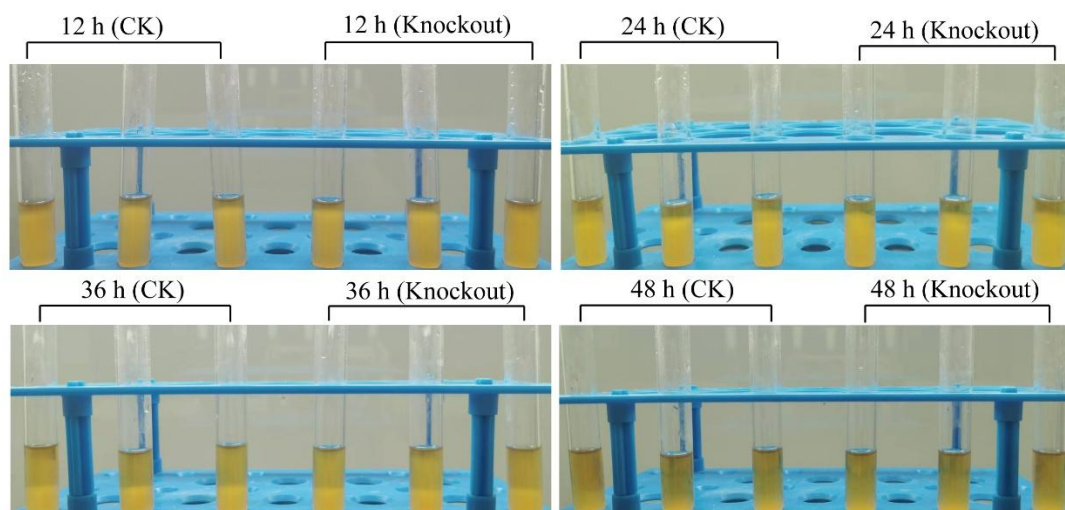


Figure S13 Effects of *rrp6* knockout on the growth of *L. plantarum* 163

Note: CK: *L. plantarum* 163; Knockout: *rrp6* gene knockout in *L. plantarum* 163.

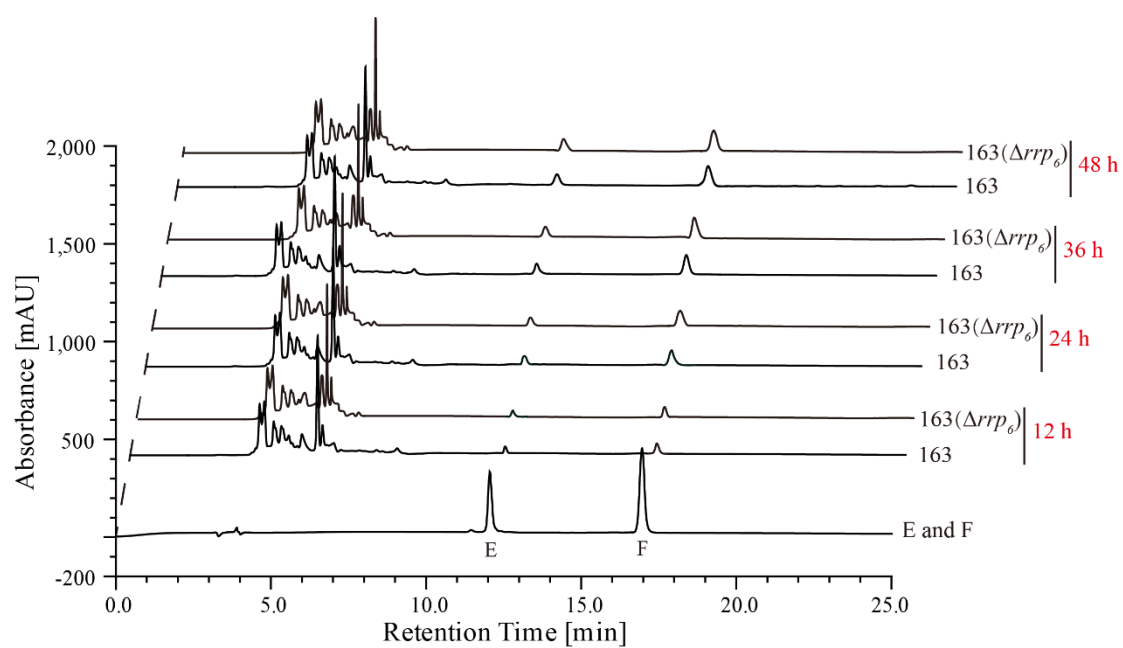


Figure S14 RP-HPLC analysis of plantaricin E and F produced by 163(Δrrp_6)

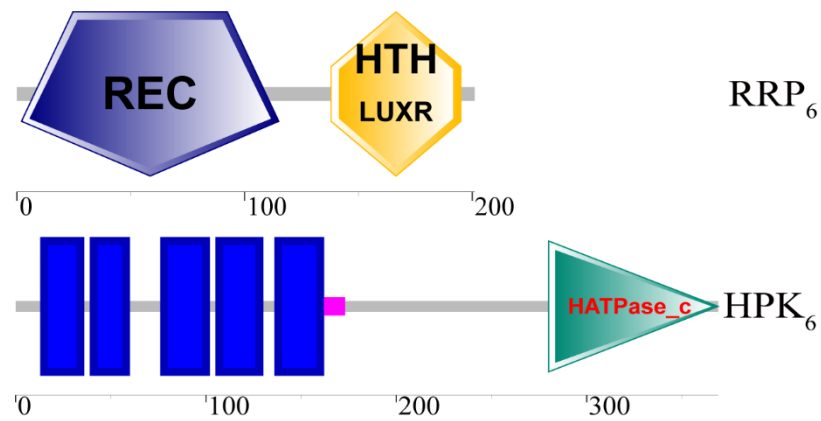


Figure S15 The domain of the two-component systems HPK_6/RRP_6 .

Note: REC: Start from 2 to 115; HTH_LUXR: Start from 138 to 195; Transmembrane region 1: Start from 15 to 34; Transmembrane region 2: Start from 41 to 58; Transmembrane region 3: Start from 78 to 100; Transmembrane region 4: Start from 107 to 128; Transmembrane region 5: Start from 138 to 160; Low complexity: Start from 162 to 173; HATPase_c: Start from 280 to 369

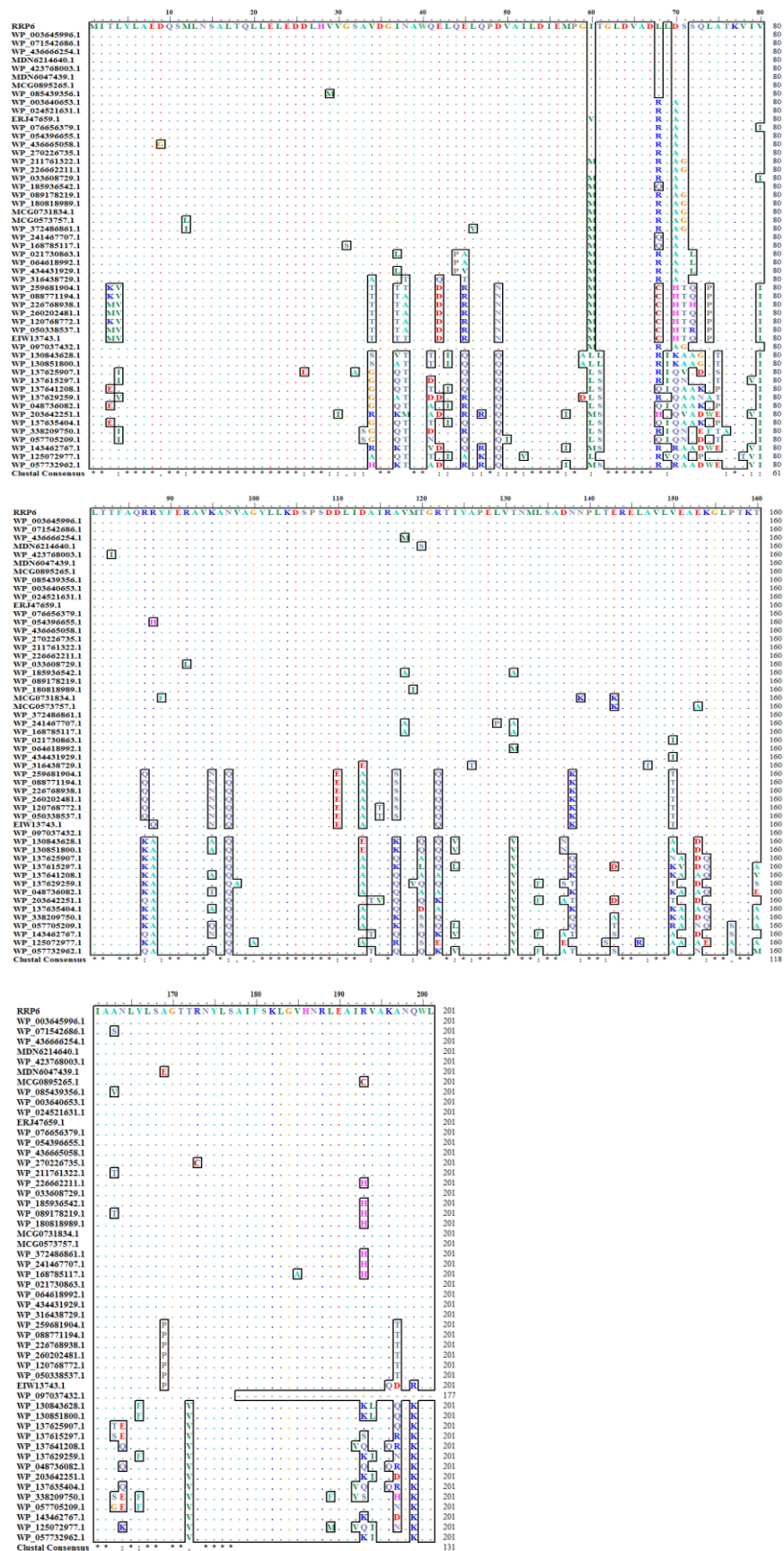


Figure S16 Multiple sequence alignment of RRP₆ homolog proteins

Table S1 Strains and plasmids presented in this study

Strains or plasmids	Characteristics	Source
Plasmids		
pET30a	T7 promoter, lac operator, Kan ^r	Novagen
pET30a- <i>rrp6</i>	pET30a with <i>rrp6</i> gene, Kan ^r	This study
pMG36e	<i>E. coli</i> and <i>L. plantarum</i> shuttle, Em ^r	Our lab
pMG36e- <i>rrp6</i>	pMG36e with <i>rrp6</i> , Em ^r	This study
pNZ5319	<i>E. coli</i> and <i>L. plantarum</i> shuttle, Cm ^r , Em ^r	Our lab
pNZ5319- <i>rrp6</i>	pNZ5319 with <i>rrp6</i> , Cm ^r , Em ^r	This study
Strains		
<i>E. coli</i> DH5α	F-φ80 lac ZΔM15 Δ(lacZYA-arg F) U169 endA1 recA1 hsdR17(rk ⁻ ,mk ⁺) supE44λ- thi-1 gyrA96 relA1 phoA	TransGen Biotech
<i>E. coli</i> BL21(DE3)	F ⁻ dcm ompT hsdS (rB ⁻ mB ⁻) gal	Zoman Biotechnology, Beijing, China
<i>E. coli</i> BL21(DE3)(<i>rrp6</i>)	F ⁻ dcm ompT hsdS (rB ⁻ mB ⁻) gal, pET30a with <i>rrp6</i> gene, Kan ^r	This study
<i>L. plantarum</i> 163	wild-type strain, producer of plantaricin EF	Our lab
<i>L. plantarum</i> 163(<i>rrp6</i>)	<i>rrp6</i> overexpression, derivative of strain 163, Em ^r	This study
Kan ^r : kanamycin resistance; Em ^r : erythromycin resistance; Cm ^r : chloramphenicol resistance		

Table S2 Primers used in this study

Plasmid name	Primer name	Sequence 5'-3'
pMG36e	e- <i>rrp6</i> F	agcagaaaaattcgtaattcATGATAACTTTTATACTTAGCAGAAGACCAA
	e- <i>rrp6</i> R	tccaaatatcgtagcgccggTTACAACCACTGATTAGCTTTTCGC
	e-F	CCGGCGCTACGATATTTGG
	e-R	GAATTACGAATTTTTCTGCTGAAACG
pET30a	a- <i>rrp6</i> F	aactttaagaaggagatataATGATAACTTTTATACTTAGCAGAAGACCAA
	a- <i>rrp6</i> R	agtgcggccgcaagcttgcTTACAACCACTGATTAGCTTTTCGC
	a-F	GACAAGCTTGCGGCCGCA
	a-R	TATATCTCCTTCTTAAAGTTAAACAAAATTATTT
pNZ5319	9- <i>rrp6</i> F	attaaaggaccgataacgcgTTGGCTTTGGCTATTGCTTGT
	9- <i>rrp6</i> R	tggcttctgttctatcaggAACATTGATCCCCGCCAAC
	9-F	CCTGATAGAAACAGAAGCCACTGG
	9-R	CGCGTTATCGGTCCTTTAATTG

Table S3 qPCR primers used in this study

Gene name	Primer name	Sequence 5'-3'
<i>16s rRNA</i>	16s rRNA-F	GCATTAAGCATTCCGCCTGG
	16s rRNA-R	ACCTGTATCCATGTCCCCGA
<i>plnG1</i>	<i>plnG1</i> -F	AACGCATGTCGTGATTGCTG
	<i>plnG1</i> -R	CGGTGCAAAGAAAAGGGCAA
<i>plnG2</i>	<i>plnG2</i> -F	GACGAGTGGACTGGATGCAA
	<i>plnG2</i> -R	GACCCGACCATCATGCAGAA
<i>plnH</i>	<i>plnH</i> -F	CGAAGACCGCACAACAAGTG
	<i>plnH</i> -R	TGGCCTCCTTTGACTTAGCG
<i>plnST</i>	<i>plnST</i> -F	AAGCACCGCTTCCCATGATT
	<i>plnST</i> -R	ACGGCTCCCAATTTGCATCA
<i>plnU</i>	<i>plnU</i> -F	TGGTCAATGATGGGGCTGAC
	<i>plnU</i> -R	ACGGGGCTAACGATGTTTGT
<i>plnV</i>	<i>plnV</i> -F	TTTTGCACCGATTGTGCGAGG
	<i>plnV</i> -R	ACTAAAACCGCCACGCAGTA
<i>plnW</i>	<i>plnW</i> -F	ATCGGCGCAGTCATTATCGT
	<i>plnW</i> -R	GGCATCGAGTGTCATCGCTA