# Supplemental data

Identification of a strong quorum sensing- and thermo-regulated promoter for the biosynthesis of a new metabolite pesticide phenazine-1-carboxamide in *Pseudomonas* strain PA1201

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- Supplemental Figures S1-S8
- Supplemental Tables S1-S2

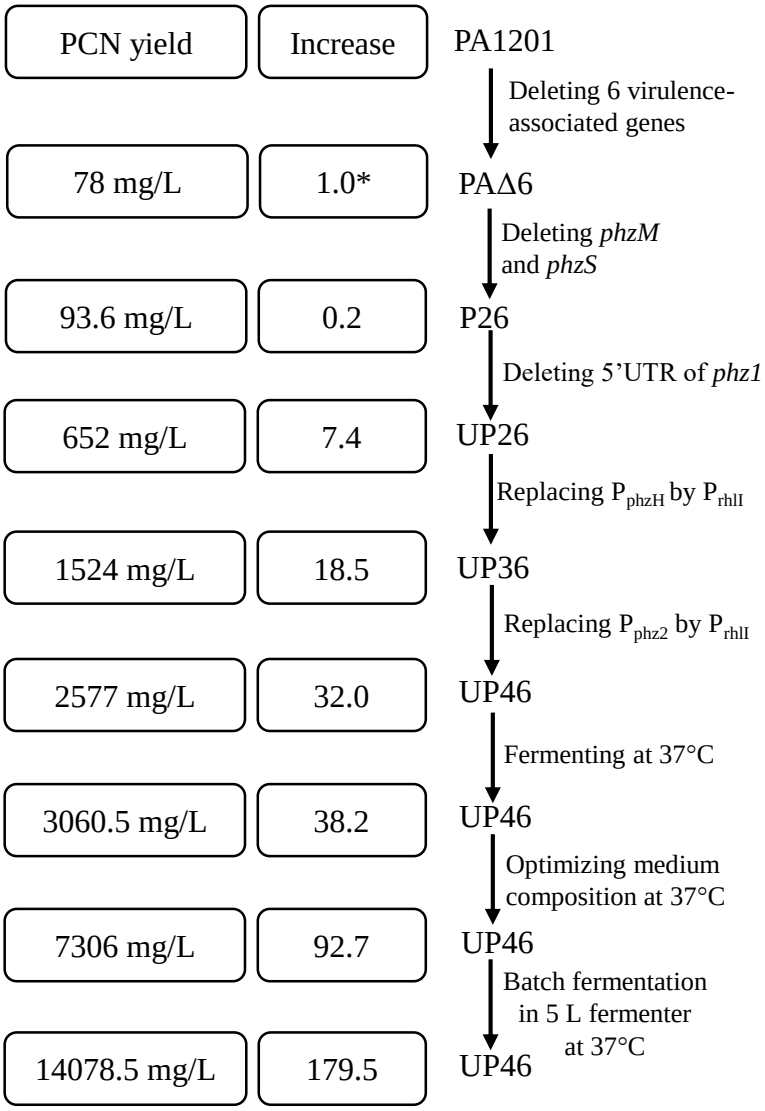



**Figure. S1**. A summary of the steps in the genetic and metabolic engineering, and fermentation condition optimization for PCN production. All the fermentation titers shown indicate those determined at 72 hour post inoculation (psi).

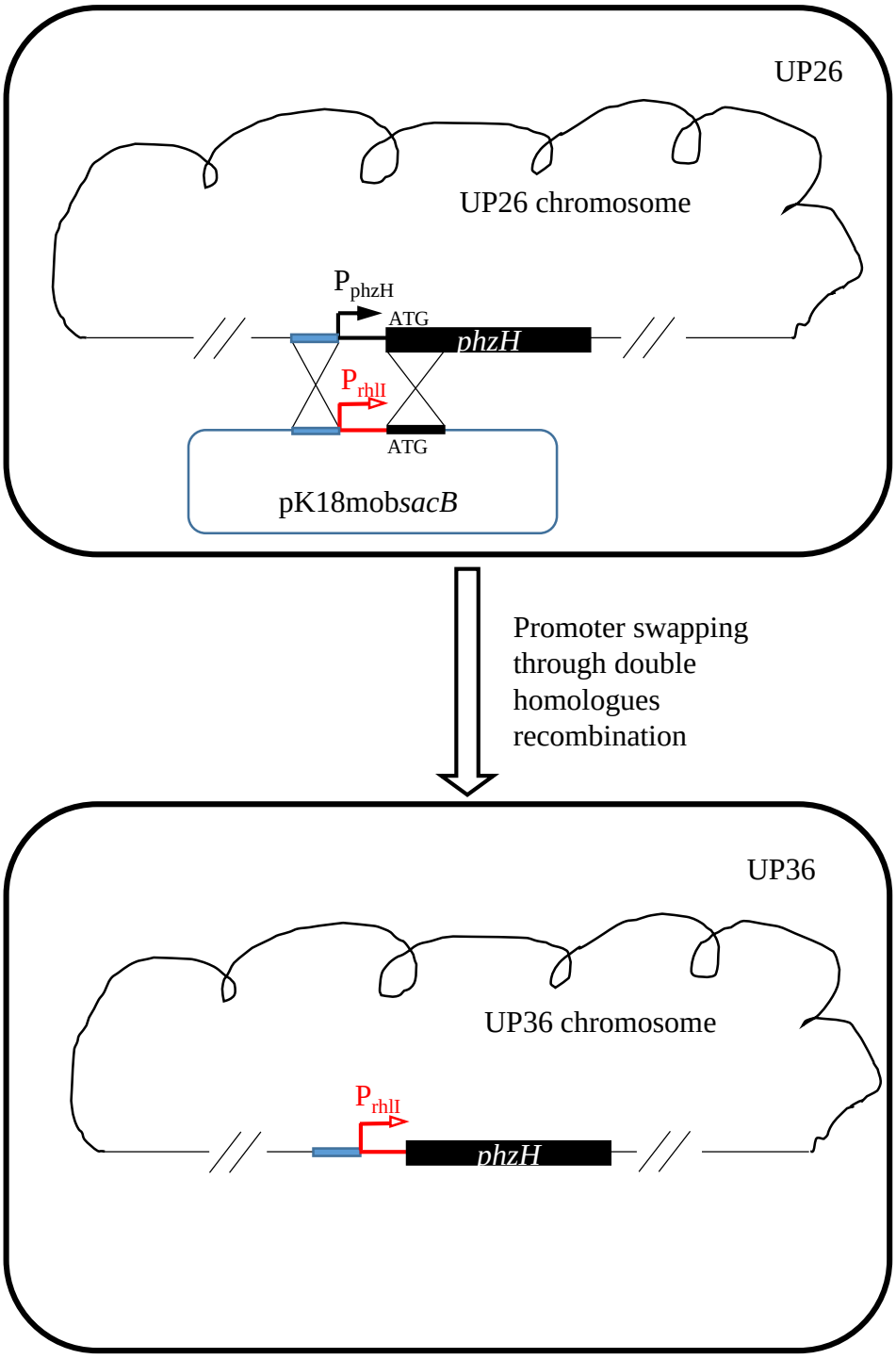



**Figure.S2**. Schematic diagram showing the principles and general steps of promoter swapping in PA1201. For details, please refer to the text.

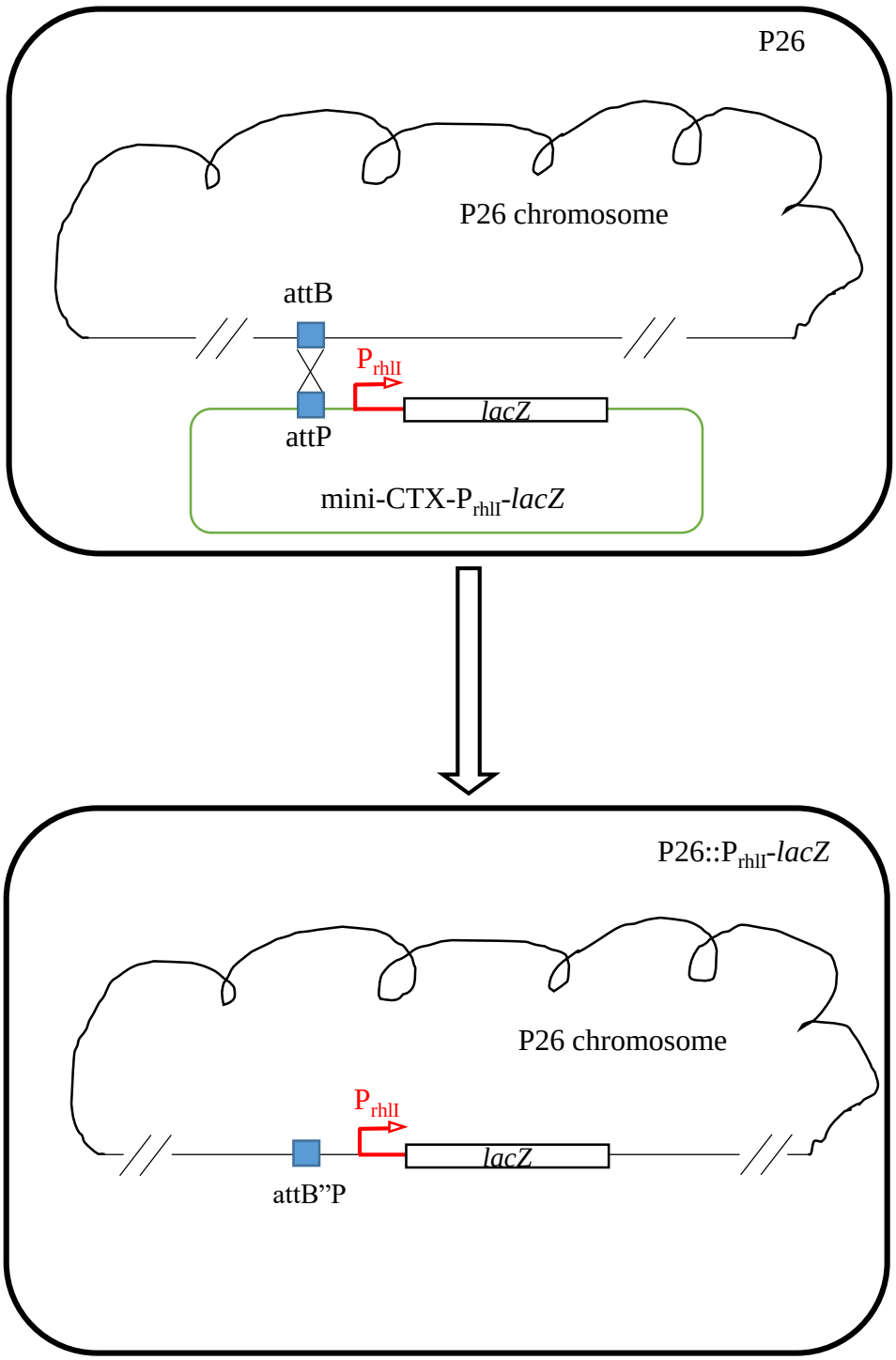
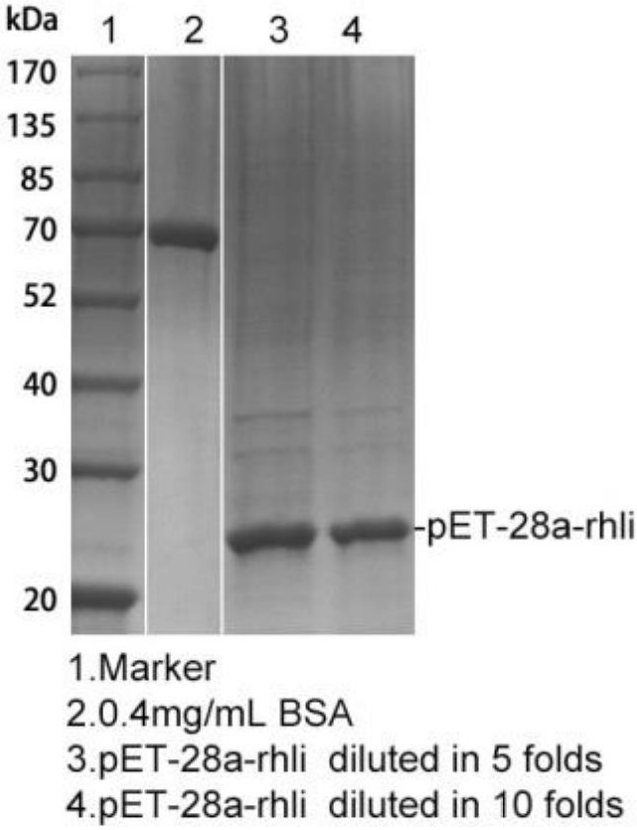



**Figure.S3**. Schematic diagram for constructing the promoter-*lacZ* fusion reporter strain. For details, please refer to Becher and Schweizer (2000).

| Gene ID | M18WT-RPKM | phz12-RPKM | MS-RPKM | MSoxyR-RPKM |
|---|---|---|---|---|
| PM18_00040 | 6379.976994 | 7244.560787 | 9522.300375 | 10472.84027 |
| PM18_01222 | 18275.65688 | 16253.09475 | 17561.8563 | 25697.8078 |
| PM18_01568 | 45989.20172 | 32490.98734 | 46885.41377 | 61470.05883 |
| PM18_01569 | 5091.544712 | 7238.403871 | 29335.84305 | 16452.71085 |
| PM18_01639 | 9648.689187 | 23560.11663 | 47853.53837 | 48928.92109 |
| PM18_01929 | 7356.138428 | 6897.35394 | 5352.263849 | 5579.230851 |
| PM18_03588 | 68002.64641 | 57712.84447 | 34686.65367 | 44408.75635 |
| PM18_01488 | 13039.86247 | 9081.454589 | 79848.85139 | 98557.57902 |
| PM18_01489 | 13694.48381 | 10660.43852 | 12430.71215 | 16928.29955 |
| PM18_03269 | 13740.27094 | 14752.47108 | 20910.4925 | 22762.19611 |
| PM18_03455 | 20472.89751 | 18233.57846 | 9246.088675 | 11378.38565 |
| PM18_02110 | 7095.998323 | 8738.352631 | 15686.50718 | 13619.89129 |
| PM18_04052 | 8475.223205 | 7565.221173 | 13188.97446 | 10838.02456 |
| PM18_04188 | 6519.310193 | 6627.412869 | 19215.71016 | 20379.3727 |
| PM18_04554 | 10764.07068 | 10938.22009 | 5254.248274 | 5081.589009 |
| PM18_04701 | 19423.3805 | 18142.05105 | 8090.597595 | 9122.663681 |
| PM18_04735 | 9546.425824 | 8018.128833 | 10626.40394 | 7019.391193 |
| PM18_04846 | 3925.956993 | 3513.977172 | 17763.52776 | 16153.36257 |
| PM18_04847 | 6582.894004 | 5853.03782 | 18688.67856 | 16166.66136 |

**Figure. S4**. The genes with RPKM >5000 identified by RNA-Seq analysis of the four strains, PA1201, Δ*phz1*Δ*phz2*, Δ*rsaL* and Δ*oxyR*. RNA, which were described in Jin et al. (2015), Sun et al. (2017) and Sun et al. (2020).

A




B

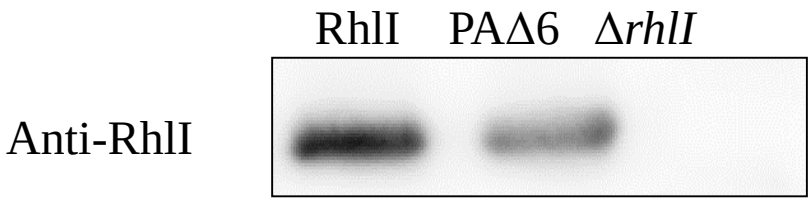



**Figure. S5**. Verification of the generated polyclonal antibody against RhlI protein. (A) Expression and purification of RhlI protein. (B) Western blotting analysis of purified RhlI and total proteins from the strains PAΔ6 and Δ*rhlI*.

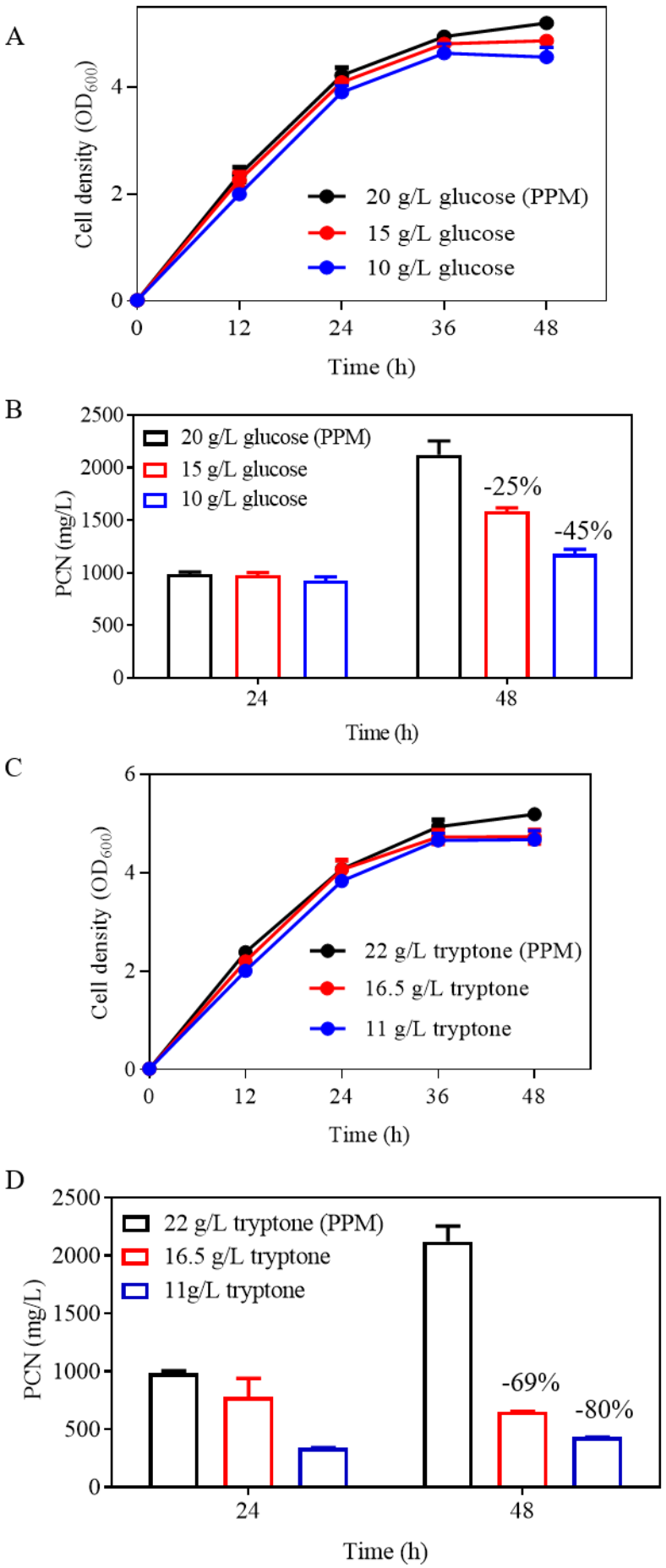



**Figure. S6.** Effects of glucose and tryptone on bacterial growth and PCN production. (A) Bacterial growth of UP46 in the presence of 10 to 20 g/L glucose. (B) PCN production of UP46 in the presence of 10 to 20 g/L glucose. (C) Bacterial growth of UP46 in the presence of 11 to 22 g/L tryptone. (D) PCN production of UP46 in the presence of 11 to 22 g/L tryptone

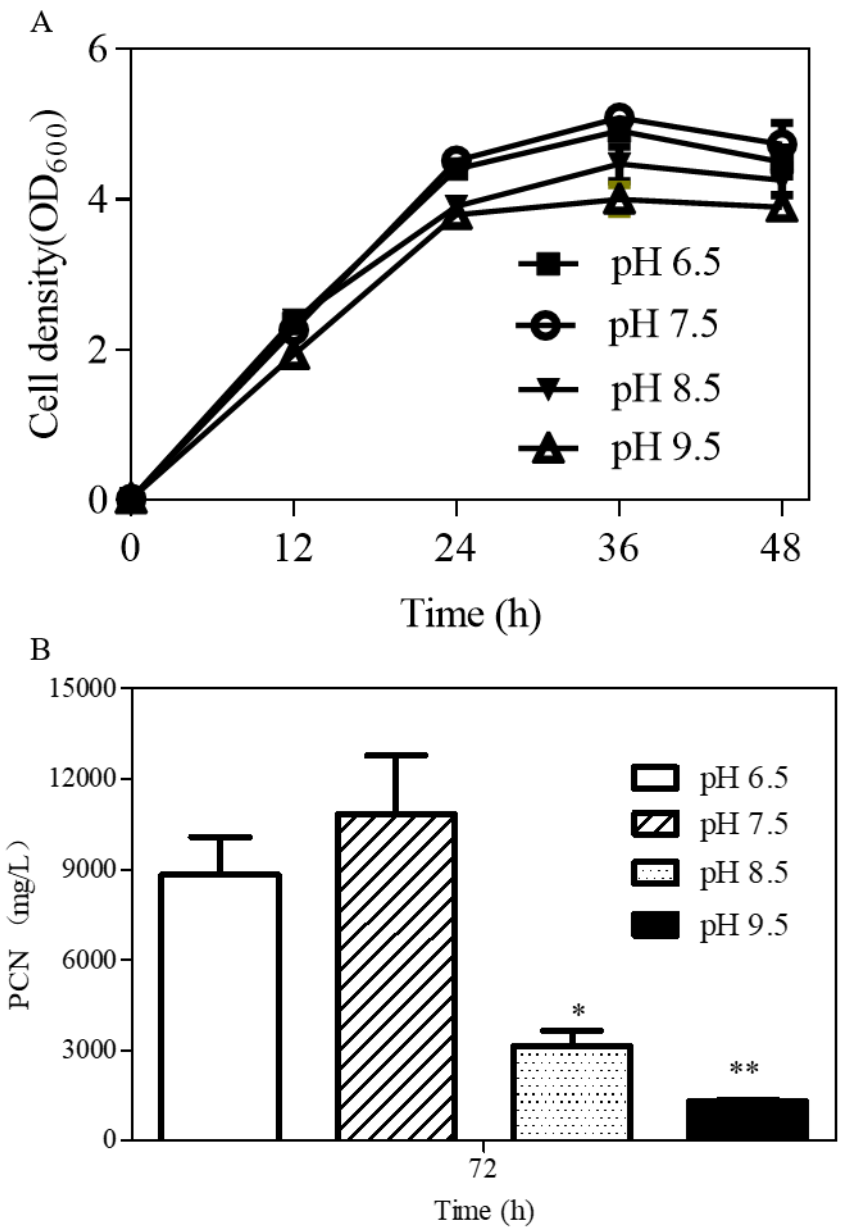



**Figure. S7**. Effects of pH values on the growth and PCN production of strain UP46 in PPN medium at 37°C. (A) Growth curves of UP46. (B) PCN production of strain UP46. Shown are averages for three technical repeats with standard deviation. Statistically significant differences with respect to PPM medium are indicated by one or more asterisks.

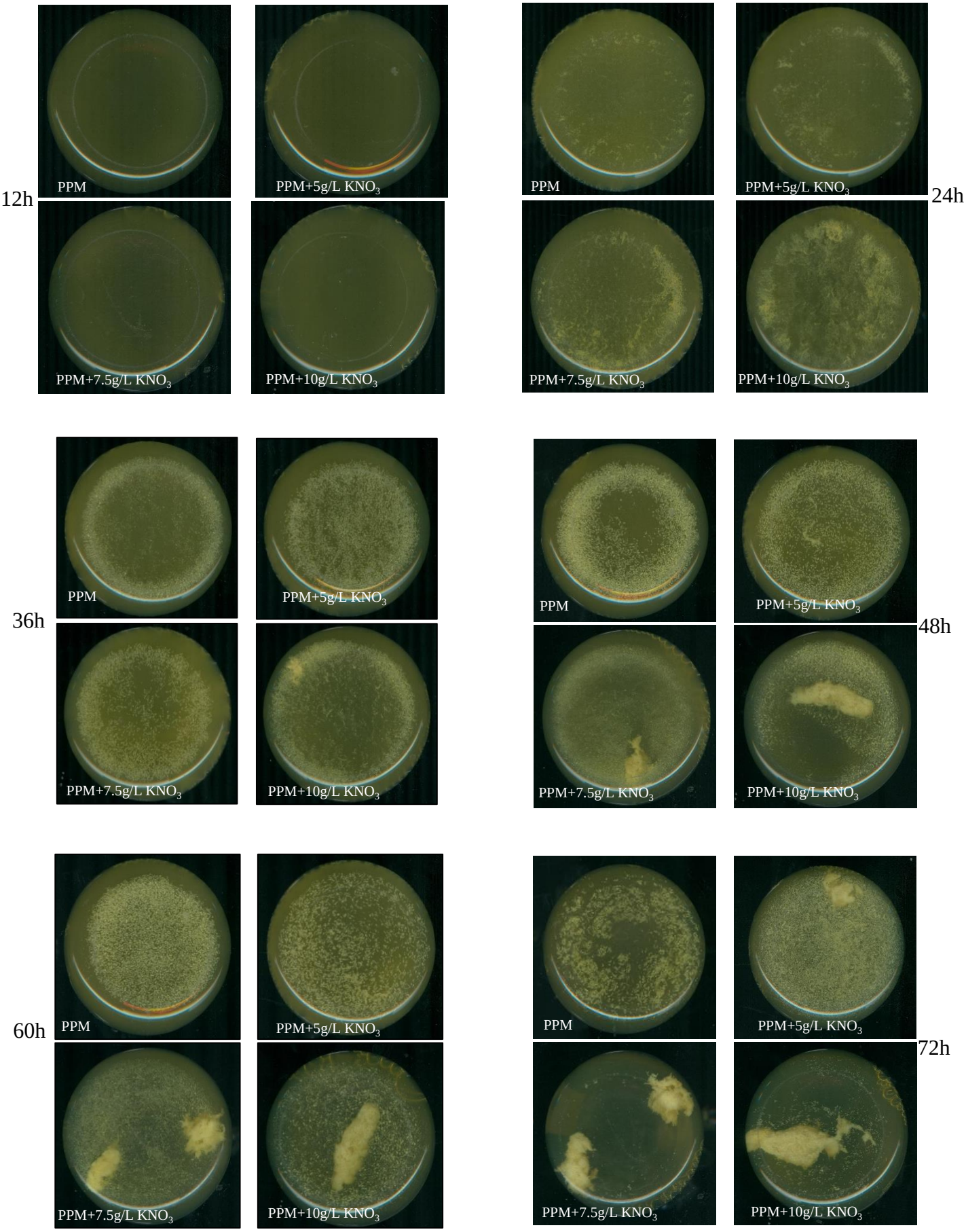



**Figure.S8**. Aggregates formed when strain UP46 was grown in 50 ml PPM media containing 5 to 15 g/L $KNO_3$ at 37 °C in a 250 ml flask.

**Table S1** Strains used in this study

| Strains | Relevant characteristics | Source |
|---|---|---|
| DH5α | *E. coli F– Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1 phoA* supE44 thi-1 gyrA96 relA1 | lab stock |
| S17-1 λpir | *E. coli* recA pro(RP4-2 Tet::Mu Kan::Tn7) | lab stock |
| PA1201 | *P. aeruginosa* wild-type strain, $Spe^R$ | lab stock |
| PAΔ6 | *exsA*, *toxA* and *adhesin*; *pilA-D*, *pilG-K* and *hcnABC* clusters in-frame deletion mutant of PA1201 | lab stock |
| pRK2013 | A conjugative plasmid, $Kan^R$ | lab stock |
| P26 | The *phzM, phzS* in-frame deletion mutant of PAΔ6, $Spe^R$ | This study |
| UP26 | 5'UTR of *phzA1-G1* deletion mutant of P26, $Spe^R$ | This study |
| UP26::*lacZ* | Single copy insertion of mini-CTX-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | |
| UP26::$P_{lasB}$-*lacZ* | Single copy insertion of $P_{lasB}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| UP26::$P_{rhlI}$-*lacZ* | Single copy insertion of $P_{rhlI}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| UP26::$P_{rhlR}$-*lacZ* | Single copy insertion of $P_{rhlR}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| UP26::$P_{pqsA}$-*lacZ* | Single copy insertion of $P_{pqsA}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| UP26::$P_{oprI}$-*lacZ* | Single copy insertion of $P_{oprI}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| UP26::$P_{phzH}$-*lacZ* | Single copy insertion of $P_{phzH}$-*lacZ* in the UP26 chromosomal attB, $Tet^R$ | This study |
| Δ*phzH* | The *phzH* in-frame deletion mutant of P26, $Spe^R$ | lab stock |
| Δ*phzH*(pBBR) | The Δ*phzH* strain harboring the plasmid pBBR, $Kan^R$ | This study |
| Δ*phzH*($ATG_{-1}$) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-$ATG_{-1}$, $Kan^R$ | This study |
| Δ*phzH*(ATG) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-ATG, $Kan^R$ | This study |
| Δ*phzH*($ATG_{+1}$) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-$ATG_{+1}$, $Kan^R$ | This study |
| Δ*phzH*(TGT) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-TGT , $Kan^R$ | This study |
| Δ*phzH*($P_{108}$) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-$P_{108}$, $Kan^R$ | This study |
| Δ*phzH*($P_{180}$) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-$P_{180}$, $Kan^R$ | This study |
| Δ*phzH*($P_{504}$) | The Δ*phzH* strain harboring the *phzH* expression plasmid pBBR-$P_{504}$, $Kan^R$ | This study |
| UP36 | Swapping *phzH* promoter with $P_{rhlI}$ mutant of UP26, $Spe^R$ | This study |
| UP46 | Swapping *phzA2-G2* promoter with $P_{rhlI}$ mutant of UP36, $Spe^R$ | This study |

**Table S2**. Primers and plasmids used in this study

| Application | Oligos and sequence(5'to3') |
|---|---|
| Deletion of *phzS* | *phzS*-F1: CCCAAGCTTAGCCGCGGCCCATTCCTCTTTTTC |
| | *phzS*-R1: CCGTTCTTCGCCGACTGGACCTG |
| | *phzS* -F2: CCAGTCGGCGAAGAACGGGGCGCCGACCAGCACATCG |
| | *phzS* -R2: CGGGATCCCCCCCTTGGCGTCGGCATCA |
| Deletion of *phzM* | *phzM*-F1: CGGGATCCCGGGGTGTTCTGCGGTATTTCTCG |
| | *phzM*-R1: GGCATCCGGGCAGCGCTTCAG |
| | *phzM* -F2: AAGCGCTGCCCGGATGCCGACGGCCGGGTGGTGGTGAT |
| | *phzM* -R2: GGAATTCCCTGGCGGTGCTGGAGAAC |
| Deletion of *phzH* | *phzH*-F1: CGGGATCCCGGCCGCACGGATGTTTTCAGC |
| | *phzH*-R1: CTCGCGCAGGGCATCGTGGTTGTA |
| | *phzH*-F2: CACGATGCCCTGCGCGAGAAGTTGCGCCGCTATTGGGAGGTA |
| | *phzH*-R2: GGAATTCCCATGTCGCCGAAGGTGAAAGGTA |
| Deletion of *phz1* cluster 5'UTR | UTR-F1: GGAATTCGTTTTATTTGCGGAACGGCTATTC |
| | UTR-R1: CATACCTGGAGAGCCCTCTCGG |
| | UTR -F2: GAGGGCTCTCCAGGTATGGATTGCATAAAACACAGAACGCTC |
| | UTR -R2: CCCAAGCTTAGCCCTCGACATCCCTCAGC |
| Construction of *lasB* reporter strain | $P_{lasB}$-For: CCCAAGCTTGGCGCCTGCTCTCCGATGGTCA |
| | $P_{lasB}$-Rev: CGGGATCCCTTCTTCATCTTGTTCAGTTCTCCTGG |
| Construction of *rhlI* reporter strain | $P_{rhlI}$-For: GGAATTCGAGCTCCGGGGAAATCGCCATCAT |
| | $P_{rhlI}$-Rev: CGGGATCCTTCCAGCGATTCAGAGAGCAATTCGAT |
| Construction of *rhlR* reporter strain | $P_{rhlR}$-For: GGAATTCCGCGCCACCCCAGCAGGATTG |
| | $P_{rhlR}$-Rev: CGGGATCCGCTACGCAAACCGTCCCACCACAG |
| Construction of *pqsA* reporter strain | $P_{pqsA}$-For: CCCAAGCTTGATGGCCGCCTGCTTCC |
| | $P_{pqsA}$-Rev: GGAATTCAGTCTGGCCCCGATAGTGATAAAC |
| Construction of *oprI* reporter strain | $P_{oprI}$-For: CGGAATTCCAGCTCGCCATCCGCCTCTCCT |
| | $P_{oprI}$-Rev: CGGGATCCGTTCATCGTGTTCCCCTTAAGGTGGAC |
| Construction of *phzH* reporter strain | $P_{phzH}$-For: CGGCTTCGCTGCTGCTGTGGATGT |
| | $P_{phzH}$-Rev: CCAGAGAACATGCTGAAAACAGAGAA |
| Construction of *phzH* overexpression strains | $P_{108}$-For: CGGGATCCCTGCTTATCTTCAGGTCCTCGAAAAGTTC |
| | $P_{180}$-For: CGGGATCCGATATTAATTAACTCGGCCACGTT |
| | $P_{504}$-For: CGGGATCCCATTCCCCGCGCCGCCCTACTTTT |
| | $ATG_{-1}$-For: CGGGATCCATGACCGATACGCTCGCCTTGCGC |
| | ATG-For: CGGGATCCATGTGCGGTCTCGCGGGTTGG |
| | $ATG_{+1}$-For: CGGGATCCATGAGTTTCCCGATAAACATCAATT |
| | TGT-For: CGGGATCCTGTTTTCAGCATGTTCTCTGGATGAGTT |
| | *phzH*-Rev: CCCAAGCTTTCAGGCGGAGAGCCCGTACAAC |
| Relative *phzH* fold | qPCR-F: CATAGGGAAACTCCTATAATTGATGTTTATC |
| | qPCR-R: ACTCGGCCACGTTTTTTCCTGTTCTA |

Table S2. Primers and plasmids used in this study (continued)

| Application | Oligos and sequence(5'to3') |
|---|---|
| Confirming promoter reporter strains | Pser-up: CGAGTGGTTTAAGGCAACGGTCTTGA |
| | Pser-down: AGTTCGGCCTGGTGGAACAACTCG |
| | *lacZ*-For: GCTCCGCCGCCTTCATACTGC |
| | *lacZ*-Rev: AGCGCCGAAATCCCGAATCTCT |
| *phzH* promoter swapping | $P_{rhlI}$::*phzH*-F1: CGGGATCCGGCTTCGCTGCTGCTGTGGATGT |
| | $P_{rhlI}$::*phzH*-R1: ATTTCCCCGGAGCTCCGGCGCGGGGAATGGAGAA |
| | $P_{rhlI}$::*phzH*-F2: GAGCTCCGGGGAAATCGCCATCAT |
| | $P_{rhlI}$::*phzH*-R2: CGCGAGACCGCACATGACCAAGTCCCCGTGTCGTGCC |
| | $P_{rhlI}$::*phzH*-F3: ATGTGCGGTCTCGCGGGTTGG |
| | $P_{rhlI}$::*phzH*-R3: CCCAAGCTTGCGGAACGGCGTCTGCGAAGT |
| | $P_{rhlI}$::*phzH*39-R2: TATCGGGAAACTCATGACCAAGTCCCCGTGTCGTGCC |
| | $P_{rhlI}$::*phzH*39-F3: ATGAGTTTCCCGATAAACATCAATTATAGG |
| *phz2* promoter swapping | $P_{rhlI}$::*phz2*-F1: GCTCTAGAGAACCGTTTCTTCTGGGAGGAAGCGC |
| | $P_{rhlI}$::*phz2*-R1: CTAGGCGACGAGGCGTCGATTCAGT |
| | $P_{rhlI}$::*phz2*-F2: GACGCCTCGTCGCCTAGGAGCTCCGGGGAAATCGCCATCAT |
| | $P_{rhlI}$::*phz2*-R2: GACCAAGTCCCCGTGTCGTGCC |
| | $P_{rhlI}$::*phz2*-F3: GACACGGGGACTTGGTCATGCGAGAGTACCAACGGTTGAAAGGG |
| | $P_{rhlI}$::*phz2*-R3: CGGAATTCGGCATTATCGAGCATCGTCATATCTCCTC |

| Plasmids | **Relevant characteristics** | **Source** |
|---|---|---|
| pK18mobs*acB* | Broad-host-range gene swapping vector, *sacB*, $Kan^R$ | lab stock |
| pBBR1MCS | The broad-host-range cloning vector with bule-white selection marker, $Gm^R$ | lab stock |
| pK18-*phzM* | pK18 containing *phzM* cluster flanking region, $Kan^R$ | lab stock |
| pK18-*phzS* | pK18 containing *phzS* cluster flanking region, $Kan^R$ | lab stock |
| pK18-*phzH* | pK18 containing *phzH* cluster flanking region, $Kan^R$ | lab stock |
| pBBR-$P_{108}$ | pBBR containing $P_{108}$ coding sequence, $Kan^R$ | This study |
| pBBR-$P_{180}$ | pBBR containing $P_{180}$ coding sequence, $Kan^R$ | This study |
| pBBR-$P_{504}$ | pBBR containing $P_{504}$ coding sequence, $Kan^R$ | This study |
| pBBR-$ATG_{-1}$ | pBBR containing $ATG_{-1}$ coding sequence, $Kan^R$ | This study |
| pBBR-ATG | pBBR containing ATG coding sequence, $Kan^R$ | This study |
| pBBR-$ATG_{+1}$ | pBBR containing $ATG_{+1}$ coding sequence, $Kan^R$ | This study |
| pBBR-TGT | pBBR containing TGT coding sequence, $Kan^R$ | This study |
| mini-CTX-*lacZ* | Integration-proficient vector for chromosomal insertion at the attB site, $Tet^R$ | lab stock |
| $P_{lasB}$-*lacZ* | Integration-proficient vector with the promoter region of *lasB*, $Tet^R$ | This study |
| $P_{rhlI}$-*lacZ* | Integration-proficient vector with the promoter region of *rhlI*, $Tet^R$ | This study |
| $P_{rhlR}$-*lacZ* | Integration-proficient vector with the promoter region of *rhlR*, $Tet^R$ | This study |
| $P_{pqsA}$-*lacZ* | Integration-proficient vector with the promoter region of *pqsA*, $Tet^R$ | This study |
| $P_{oprI}$-*lacZ* | Integration-proficient vector with the promoter region of *oprI*, $Tet^R$ | This study |
| $P_{phzH}$-*lacZ* | Integration-proficient vector with the promoter region of *pqsH*, $Tet^R$ | This study |
| pK18-UTR | 5'UTR of *phzA1-G1* deletion region, $Kan^R$ | This study |
| pK18-$P_{rhlI}$-*phzH* | pK18 containing $P_{rhlI}$ and *phzH* fusion product, $Kan^R$ | This study |
| pK18-$P_{rhlI}$-*phz2* | pK18 containing $P_{rhlI}$ and *phz2* fusion product, $Kan^R$ | This study |