Supporting Information for

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Cinnamaldehyde attenuates intergeneric horizontal transfer of antibiotic resistance genes by disrupting quorum sensing

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Text. S1 Conjugative transfer experiments

For the conjugation experiment, the donor and recipient bacteria were mixed at a ratio of 1:1, and cinnamaldehyde solution with the final concentration of 10 and 100 mg/L was added to the conjugation system. All the mixtures were mated at 37 °C for 6 hours without shaking. Then, the bacteria in the conjugation system were diluted to an appropriate concentration before spread on the Pseudomonas Isolation Agar selection plates to screen the recipients, and Pseudomonas Isolation Agar selection plates containing Amp, Tet, Km to select PAO1 transconjugants that acquired the RP4 plasmid. All the plates were incubated at 37 °C for 18-24 hours until counting the number of transconjugants and recipients. All conjugative transfer experiments were conducted in triplicate or more. The conjugative transfer frequency was calculated using the following formula:

$$\text{Conjugative transfer frequency} = \frac{\text{Number of transconjugants (CFU/mL)}}{\text{Number of recipients (CFU/mL)}}$$

Text. S2 RNA extraction, cDNA synthesis, and RT-qPCR

Total RNA was extracted from the cinnamaldehyde-treated conjugation system under LB medium environment using an RNA prep pure Cell/Bacteria Kit (TIANGEN, Beijing, China) according to the manufacturer’s instructions. Subsequently, the RNA was reverse transcribed into cDNA using the M5 Super plus qPCR RT kit with gDNA remover (MF166-plus-01, Mei5bio, Beijing, China). The relative expression levels of QS-related genes (rhlI and rhlR) were quantified using Applied Biosystems Quantstudio 5 Real-Time PCR System. The rpoD gene was used as an internal reference gene for normalization, and the 2-△△CT calculation method was used for quantitative analysis of the target gene.

The qRT-PCR was performed using the SYBR® Green Premix Pro Taq HS qPCR Kit II (Rox Plus) (AG11719, Hunan, China) on a Thermo QuantStudio 5 real-time PCR system. For each real-time PCR reaction, a mixture was prepared comprising 10 μL of 2X SYBR® Green Pro Taq HS Premix II (ROX plus), 0.4 μL of forward and reverse primer, 2 μL of cDNA template, and 7.2 μL of RNase-free water. The amplification process consisted of an initial step at 95 °C for 30 seconds, followed by 40 cycles of denaturation at 95 °C for 5 seconds, annealing at 60 °C for 10 seconds, and then extension at 72 °C for 20 seconds. Each experiment was performed with a minimum of three replicates.

Text. S3 PAO1 biofilm determination

Specifically, the bacterial liquid in the cultured well plate was removed carefully, leaving the biofilm on the well wall. Each well was washed twice with PBS buffer to remove unadsorbed bacteria. After drying, 200 μL of methanol was added to fix the biofilm for 15 minutes and then the methanol was discarded. Then, 200 μL of 0.1% (v/v) crystal violet was added to each well to stain the fixed biofilm for 30 minutes in the dark at room temperature. Excess dye was washed off with sterile water. Finally, 200 μL of 33% acetic acid (v/v) solution was used to dissolve the attached crystal violet for 30 minutes at 37 °C. The biofilm biomass was quantified by measuring the absorbance of each well at 590 nm using a microplate spectrophotometer. All experiments were conducted in triplicate or more.

Text. S4 Rhamnolipid determination

Briefly, the cultured bacteria were centrifuged at 12000 rpm for 10 minutes, and the supernatant was adjusted to its pH of 2.0. Then, 1 mL of ethyl acetate was added and vortexed to mix well. Allowed the samples to stratify before transferring the organic layer to a new centrifuge tube. After vacuum drying, 500 μL of DI water was added to redissolve. Then, 100 μL of the dissolved sample and the prepared 0.19% orcinol sulfuric acid solution were mixed in an ice-water bath and heated at 80 °C for 30 minutes. The absorbance of mixtures was determined at 410 nm to measure the rhamnolipid content. Each experiment was repeated at least three times.

Table S1. Primers were used for qPCR amplifications in this study.

Gene	Primer		
	Sequence of the primer	Target gene	Product size (bp)
rhlI-F	GCAGCTGGCGATGAAGATATTC	*rhlI*	62
rhlI-R	CGAACGAAATAGCGCTCCAT		
rhlR-F	AACGCGAGATCCTGCAATG	*rhlR*	77
rhlR-R	GCGCGTCGAACTTCTTCTG		
rpoD-F	CGCCCAGGTGCGAATC	*rpoD*	87
rpoD-R	ACAAGATCCGCAAGGTACTGAAG		