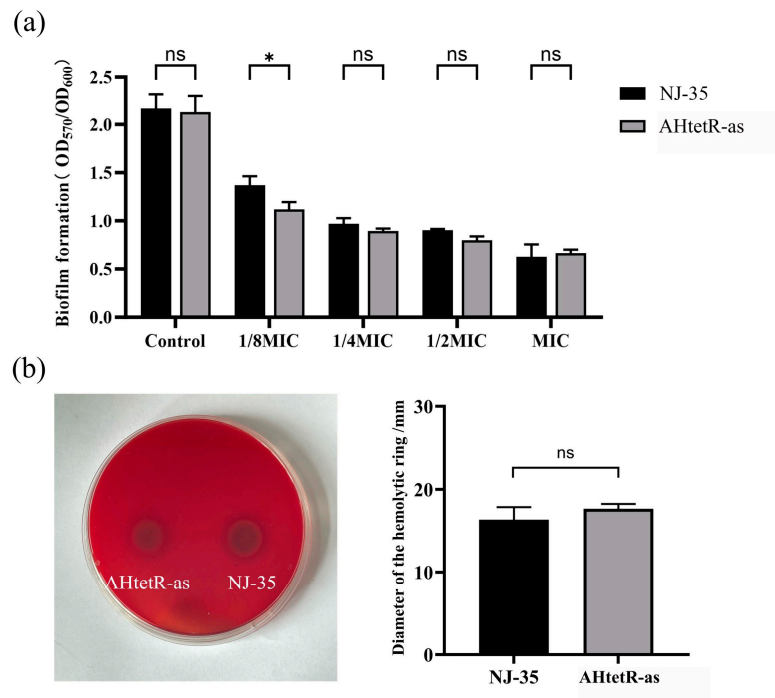


Supplement Materials

Supplement Figure

Figure S1 Effects of *tetR* knockdown on virulence phenotypes of *A. hydrophila*. (a) Biofilm formation ability. (b) Hemolytic activity. The values were presented as mean $\pm$ SD and the significance was determined by *t*-test (* $P < 0.05$; ns $P > 0.05$).



Supplement Table

Table S1 Prokaryotic transcriptome data analysis method

Category	Analysis Content	Tools/Databases Used	Version and References
Data filtering	Filtering of raw data from Illumina platform	FASTP	(version 0.20.0) [1]
	Clean reads were mapped to the reference genome and reads mapped to ribosome RNA were removed	Bowtie2	(version 2.2.8) [2]
	Calculated gene expression	RSEM	(version:1.2.19) [3]
Analysis of samples correlation and principle component	The correlation coefficient among samples was calculated.	R package psych	(version 1.8.4) [4]
Analysis of differentially expressed genes	Identify differentially expressed genes (DEGs) among samples with fold change ≥ 2 and FDR < 0.05. DEGs were used to analyze GO and KEGG, and FDR < 0.05 was used as threshold.	edgeR package	(version 3.12.1) [5]
sRNA analysis	Predict sRNA and the removal of sequences less than 50 bp.	Rockhopper	(version 2.0.3) [6]
	Screen candidate sRNAs	sRNAMap database, Rfam database	(version 2009) (version13) [7,8]
	Predict secondary structures of sRNA	Vienna RNA packages	(version 2.3.5) [9]
Transcript structure analysis	Predict new transcripts	Rockhopper	(version 2.0.3)
	Verify candidate cis-NATs and cis-NATs with minimum free	RNAplex	(version 0.2) [10]

	energy more than -30 kcal/mol were removed.		
	Predict Shine-Dalgarno sequence and set the parameter of ribosome region length 20 bp.	RBSfinder	[11]
	Identify ρ -independent terminator	TransTermHP	(version 2.09) [12]
Trend analysis	Gene expression pattern analysis is used to cluster genes of similar expression patterns for multiple samples (at least 3 in a specific time point, space, or treatment dose). To evaluate the expression pattern of DEGs, the expression data of each sample was normalized and then clustered.	ShortTime-series Expression Miner (STEM)	(version 1.3.11)[13]
Gene Set Enrichment Analysis (GSEA)	Perform the gene set enrichment analysis in order to identify a set of genes in specific GO terms\KEGG Pathways which show significant differences between two groups.	GSEA, MSigDB	[14]

Table S2 Classification standards for bacterial susceptibility to tetracycline, doxycycline and minocycline

Antibiotic	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Tetracycline	≤ 14	15–18	≥ 19
Doxycycline	≤ 10	11–13	≥ 14
Minocycline	≤ 13	14–18	≥ 19

Note: Species-specific breakpoints for *A. hydrophila* were referred to the Enterobacteriaceae criteria according to CLSI M100 Ed34 (a common practice in aquatic bacteriology).

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