New insights into virulence mechanisms of rice pathogen *Acidovorax avenae* subsp. *avenae* strain RS-1 following exposure to ß-lactam antibiotics

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Supplementary Information

Figure S1

FTIR analysis of the EPS from *Acidovorax avenae* subsp. *avenae* strain RS-1. (a) The *hcp*-mutant strain; (b) The wild type strain under exposure to Amp; (c) The wild type strain. Compared to the wild type, both the mutation of *hcp* gene and exposure to Amp caused the disappearance of one peak at 859.16 cm^{-1} representing phenyl ring substitution bands/alkenes. Each experiment was repeated three times independently with similar results.

Figure S2

Gene ontology categories for the differentially expressed genes under Amp (+) vs. Amp (-) condition of *Acidovorax avenae* subsp. *avenae* strain RS-1 transcriptome. All genes were classified into Biological process, Molecular function and Cellular component catalogs based on their gene ontology annotations.

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Expression changes of T6SS-related genes in *Acidovorax avenae* subsp. *avenae* strain RS-1. (a) Mutation of *hcp* gene; (b) Exposure to Amp. Each result represents the average of three independent determinations with similar results.

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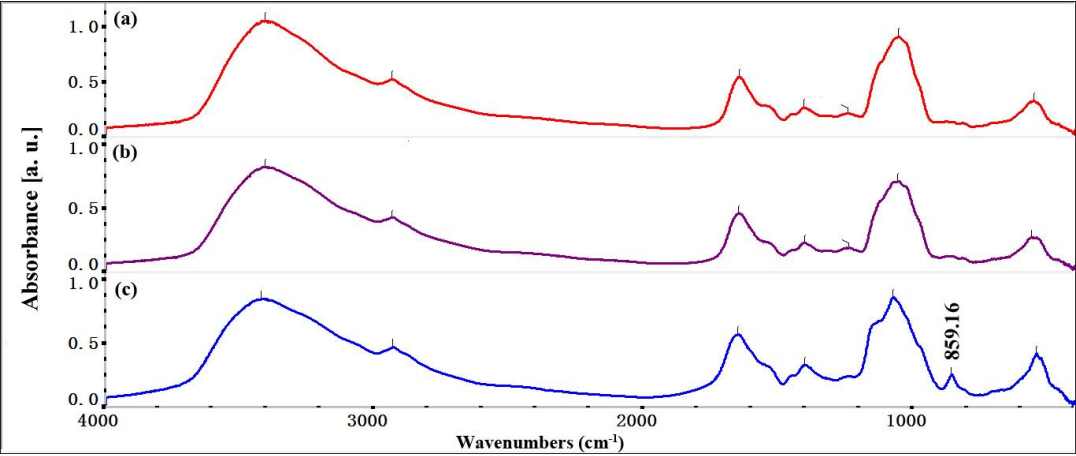
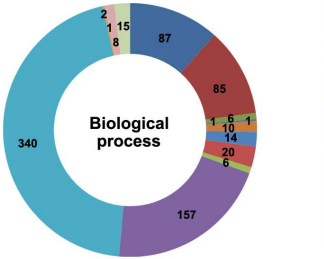
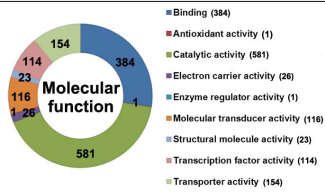
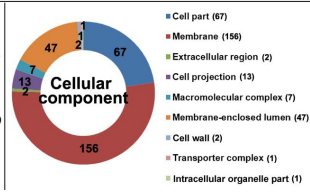
Table S12

Gene ontology enrichment analysis for differentially expressed genes under Amp (+) vs. Amp (-) condition of *Acidovorax avenae* subsp. *avenae* strain RS-1 transcriptome.


(a)
(b)
(c)
1.0
0.5
0.0
Absorbance [a. u.]
859.16
4000
3000
2000
1000
Wavenumbers (cm-1)


Biological process
87
85
1
6
10
14
20
6
157
340
2
1
15
8
Biological regulation (87)
Biosynthetic process (85)
Catabolic process (6)
Cell adhesion (1)
Cell killing (1)
Locomotion (10)
Cellular component organization or biogenesis (14)
Cellular process (20)
Establishment of localization (6)
Localization (157)
Metabolic process (340)
Negative regulation of biological process (2)
Nitrogen utilization (1)
Response to stimulus (8)
Single-multicellular process (15)


Molecular function
384
1
581
1
26
116
23
114
154
Binding (384)
Antioxidant activity (1)
Catalytic activity (581)
Electron carrier activity (26)
Enzyme regulator activity (1)
Molecular transducer activity (116)
Structural molecule activity (23)
Transcription factor activity (114)
Transporter activity (154)


Cellular component
67
156
2
13
7
47
2
1
1
Cell part (67)
Membrane (156)
Extracellular region (2)
Cell projection (13)
Macromolecular complex (7)
Membrane-enclosed lumen (47)
Cell wall (2)
Transporter complex (1)
Intracellular organelle part (1)

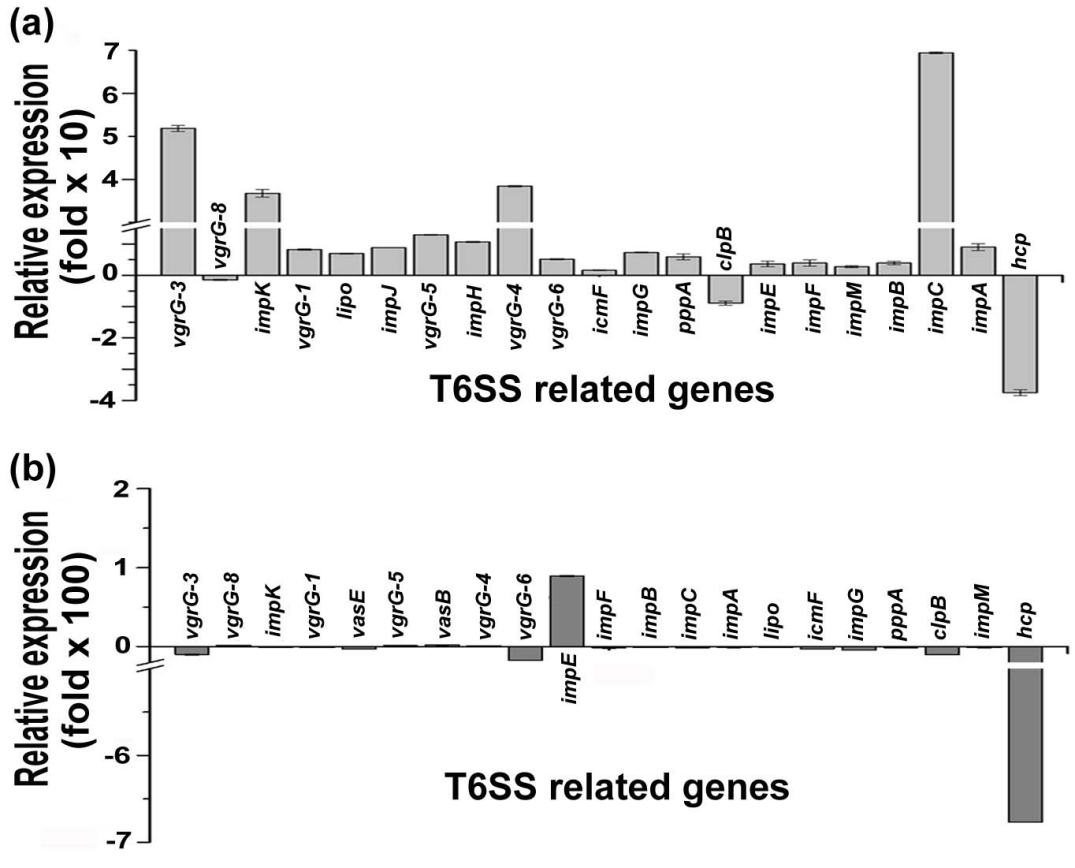

(a)
Relative expression (fold x 10)
7
6
5
4
0
-2
-4
vgrG-3
vgrG-8
impK
vgrG-1
lipo
impJ
vgrG-5
impH
vgrG-4
vgrG-6
icmF
impG
pppA
clpB
impE
impF
impM
impB
impC
impA
hcp
T6SS related genes
(b)
Relative expression (fold x 100)
2
1
0
-6
-7
vgrG-3
vgrG-8
impK
vgrG-1
vasE
vgrG-5
vasB
vgrG-4
vgrG-6
impE
impF
impB
impC
impA
lipo
icmF
impG
pppA
clpB
impM
hcp
T6SS related genes

Table S1 Primers used in this study

Name	Sequence (5'- 3')	Usage description	Sources or References
Pt-hcp	F-CCTCGGATCCATGTCCGTCGATATGTTCATG	Pull-Down and	This study
	R-GCGCGAATTCTTACATTTCCTTGTTGCCCTTG	Bacterial	
		Two-hybrid	
Pt-vgrG	F-CCGAATTCATGACACGCAGCGTCACCATAC	Pull-Down	This study
	R-CATCTCGAGGTGCACGATGAGCCCGTTCTTG		
Pt-lipo	F-GCGGATCCATGCATCGGGAAGCGATGGC	Pull-Down	This study
	R-CCGGAATTCGCGGTCTGGATCTTCACCGC		
Th-hcp	F-CCTCGGATCCATGTCCGTCGATATGTTCATG	Bacterial	This study
	R-GCGCGAATTCTTACATTTCCTTGTTGCCCTTG	Two-hybrid	
Th-lipo	F-GCGGATCCATGCATCGGGAAGCGATGGC	Bacterial	This study
	R-CCGGAATTCGCGGTCTGGATCTTCACCGC	Two-hybrid	
Th-vgrG	F-CCTCTAGAATGACACGCAGCGTCACCATAC	Bacterial	This study
	R-CCGAATTCGTGCACGATGAGCCCGTTCTTGCC	Two-hybrid	
GK-hcp-F	F-TATGGATCCATTTCCTTGTTGCCCTTG	Gene Knockout	This study
	R-CGCGAATTCCGACATCCGTTCCTTCAC		
16S	F-TTGCGGTCCCCTGCTTTCAT	Quantitative	Li et al.
rRNA	R-CGGTAACAGGTCTTCGGATGCT	Real-time PCR	(2014)[10]
clpB	F-GCAGGGCGAGAAGGACAAG	Quantitative	Li et al.
	R-GCCGAGGAACAGGAACGAG	Real-time PCR	(2014)[10]
hcp	F-CTGGGTCAGGTGGAGATCTC	Quantitative	Li et al.
	R-TGGGTCTTGATTTTGGCTGC	Real-time PCR	(2014)[10]
dotU	F-CCAGCATTACCTGCTCGAAT	Quantitative	Li et al.
	R-CCAGGTCTCGTTGTGCAGT	Real-time PCR	(2014)[10]
icmF	F-ACCGTGGGCAGCAATCTCA	Quantitative	Li et al.
	R-GCGAAGTCATCGCTCGTCA	Real-time PCR	(2014)[10]
impA	F-CTTGAACCTGCGGCGGACAC	Quantitative	Li et al.
	R-GCTCGGCGGGAATCACCAT	Real-time PCR	(2014)[10]
impB	F-ATCTCCCTCATCCTGCTCA	Quantitative	Li et al.
	R-TCAGATGCGTCCCATCAG	Real-time PCR	(2014)[10]
impC	F-GCACCACCTGGTCCACAACA	Quantitative	Li et al.
	R-CGAACTGGCCGTATTCCTCT	Real-time PCR	(2014)[10]
impE	F-TGATCGGCTCGCTGTTCG	Quantitative	Li et al.
	R-TGCTTGTACTCGCCCTTGTT	Real-time PCR	(2014)[10]
impF	F-TGGACTGGAAGGACGTGGAA	Quantitative	Li et al.
	R-AGGGTGTTGTGGTGGTTGAA	Real-time PCR	(2014)[10]
impG	F-TGGAACTTCGGCCTCTATGG	Quantitative	Li et al.
	R-TGGTGGAAGATGTCCGAGAA	Real-time PCR	(2014)[10]
impH	F-GCCACAAGTTCCTTTTGCA	Quantitative	Li et al.
	R-AAGAACGGCACGAAATCC	Real-time PCR	(2014)[10]
impJ	F-TCCAGGATGCCAACGACA	Quantitative	Li et al.
	R-GACCACGGTGGGAATGAA	Real-time PCR	(2014)[10]

impM	F-GCAATGGCGTCGTCCTCT	Quantitative	Li et al.
	R-CGGTCGTGCCGATCTTCT	Real-time PCR	(2014)[10]
lipo	F-GCAGTGCGGATGTCCGTACCTT	Quantitative	Li et al.
	R-TCCTTGCCCACCGTGATGCT	Real-time PCR	(2014)[10]
pppA	F-AGATCACGCGGGACCATT	Quantitative	Li et al.
	R-TTCCTCGTCGTCGAGCAT	Real-time PCR	(2014)[10]
vgrG-1	F-ATCCGATGGAAAAGAAACTC	Quantitative	Li et al.
	R-AATAGATGCCCTCGTGCT	Real-time PCR	(2014)[10]
vgrG-2	F-GCGTGCAATATGACGAGAGC	Quantitative	Li et al.
	R-CCGGCGGATAGAAGGGAATC	Real-time PCR	(2014)[10]
vgrG-4	F-CTGACGCAGAGCACGAAT	Quantitative	Li et al.
	R-CCGAAGCACCACATACCA	Real-time PCR	(2014)[10]
vgrG-5	F-CATCAAGACCAAGTCCAGC	Quantitative	Li et al.
	R-CAGCCATAATTGCTCTGC	Real-time PCR	(2014)[10]
vgrG-7	F-CCGATGGAAAAGAAACTCAG	Quantitative	Li et al.
	R-AATAGATGCCCTCGTGCT	Real-time PCR	(2014)[10]
vgrG-8	F-TCCTTCCAGAAGTTCAGCC	Quantitative	Li et al.
	R-GGTATTCGTCGGTCCAGATT	Real-time PCR	(2014)[10]

Table S2.

Effect of three kinds of *ß*-lactam antibiotics on the growth and pathogenicity of *Acidovorax avenae* subsp. *avenae* strain RS-1 to rice seedlings.

Treatments	Bacterial growth (OD600)			Plant parameters		
	12 h	24 h	48 h	Root length	Height	Emergence
Strain RS-1	0.15 b	0.82 c	1.24 b	15.94 b	9.16 b	23.33 c
+ Amp	0.03 d	0.79 d	1.20 b	28.97 a	21.06 a	67.74 a
+ Amo	0.03 c	0.92 b	0.97 c	38.47 a	36.63 a	40.00 b
+ Pen	0.34 a	1.12 a	1.33 a	49.10 a	43.60 a	36.67 b
None	-	-	-	177.40 a	132.47 a	100.0 a

In absence of pathogen, exposure to Amo caused a 20.05%, 40.49% and 20.00% reduction, while exposure to Pen resulted in a 48.93%, 32.79% and 20.00% reduction in root length, plant height and emergence compared to the control. However, there was no significant ($P < 0.05$) difference in plant parameter between Amp (+) and Amp (-). The same letters indicate no significant differences ($P < 0.05$) among treatments. Each treatment has 25 replicates and this experiment was repeated four times independently with similar results.

Table S3.

Summary of *Acidovorax avenae* subsp. *avenae* strain RS-1 cDNA samples sequenced using the Illumina genome analyze

Sample name	Amp (+, 1)	Amp (+, 2)
Number of reads	14,537,291	16,443,928
Mapped reads	12,679,967	15,732,263
Unique mapped	4,755,618	6,149,831
mRNA percent	35.1%	39.1%
Number and percentage at different transcript levels		
High-	809 (16.68%)	
Medium-	1088 (22.43%)	
Low-	2014 (41.52%)	
None-	940 (19.38%)	

Classification of transcription profile for various categories of transcription level was carried out as described by Nagalakshimi et al. (2008)[25]. The data of Amp (-) used as the control in this study were obtained from Li et al. (2014)[10].