Supplemental Materials for

**Implication of the type III effector RipS1 in the cool-virulence of *Ralstonia solanacearum* strain UW551**

Jeffrey K. Schachterle & Qi Huang

Contents page

**Table S1:** Bacterial Strains and plasmids used in this study

| Species/Strain | Relevant Characteristics | Source/Reference |
|---|---|---|
| *Ralstonia solanacearum* | | |
| UW551 | phy IIB seq 1 | C. Allen, USA (Gabriel et al. 2006) |
| UW551-pSIM7 | UW551 containing pSIM7; $Cm^R$ | This Work |
| UW551-TnR1 | UW551 mutant with mini Tn5 transposon inserted upstream of *ripS1* | This Work |
| UW551-TnR1-ck | Independently generated mutant of UW551 containing the mini Tn5 transposon insertion upstream of *ripS1* | This Work |
| *Escherichia coli* | | |
| S17-1 | pBAMD1-2; $Km^R$, $Amp^R$; suicide vector for transposon mutagenesis | Addgene (Martínez-García et al. 2014) |
| S17-1 | pUT-miniTn5-*luxCDABE*; $Km^R$, $Amp^R$; suicide vector for transposon mutagenesis | Hedda J. Weitz, UK (Weitz et al. 2001) |
| DH5α | pSIM7; $Cm^R$; pBBR1 origin of replication, used for counter-selection in transposon mutagenesis | Donald L. Court, USA (Datta et al. 2006) |

**Table S2:** Oligonucleotides used in this study

| Oligo Name | Sequence | Purpose of Oligonucleotide | Source/Reference |
|---|---|---|---|
| DGEN1 | GGCCACGCGTCGACTAGTCAGNNNN-NNNNNNACGCC | 1st round of arbitrary PCR, degenerate | (Miller-Williams et al. 2006) |
| DGEN2 | GGCCACGCGTCGACTAGTCAG | 2nd round of arbitrary PCR, after DGEN1 | (Miller-Williams et al. 2006) |
| MCS-Arb Ext | GGTACCGAGCTCGAATTCGCG | 1st round of arbitrary PCR from left side of transposon | This Work |
| MCS-Arb Int | CCTAGGCGGCCTGAGACACAAAG | 2nd round of arbitrary PCR and sequencing from left side of transposon | This Work |
| TDKm6 | AGTTTCATTTGATGCTCGATG | 1st round of arbitrary PCR from right side of transposon | (Mendez et al. 2018) |
| TDKm-Int | CCTTGTCTAATTAATTGCGGACCC | 2nd round of arbitrary PCR and sequencing from right side of transposon | This Work |
| *ripS1* qPCR F | AGCGCTGAACTACCCTGAGC | qPCR testing of *ripS1* expression | This Work |
| *ripS1* qPCR R | CTCATGCGCCAGAGTTCG | | This Work |
| *RRSL_04180* | ATGGACTTCATCGTGATGATGC | qPCR testing of *RRSL_04180* expression | This Work |
| *RRSL_04180* | CACGAATACGCCGACACG | | This Work |
| *ripU* qPCR F | CGTGGTGTTGAAGGAGGAAT | qPCR testing of *ripU* expression | This Work |
| *ripU* qPCR R | ATGCACATAAGCCCCTGAAC | | This Work |
| *ripW* qPCR F | CGAGTGACCATTTCCATGTG | qPCR testing of *ripW* expression | This Work |
| *ripW* qPCR F | GACCTGCTGGAGCAGTTGTA | | This Work |
| 16S qPCR F | CTAGAGTGTGTCAGAGGGAGGTAGA | qPCR testing, endogenous control | (Addy et al. 2012) |
| 16S qPCR R | ATGTCAAGGGTAGGTAAGGTTTTTC | | (Addy et al. 2012) |

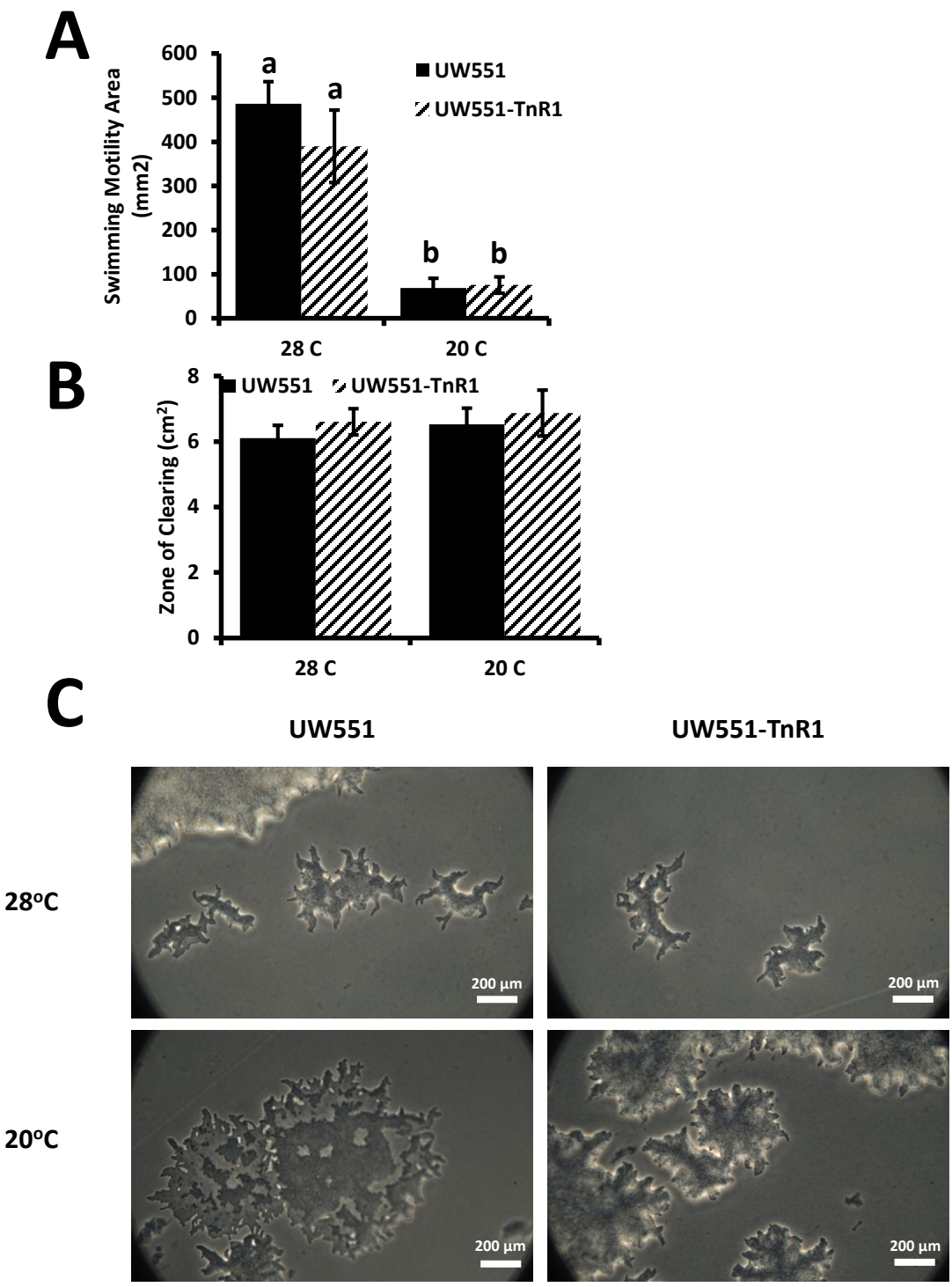


**Figure S1:** Comparison of virulence traits between *Ralstonia solanacearum* wild-type strain UW551 and mutant strain UW551-TnR1 tested at 28°C or 20°C. No significant differences were caused by the transposon insertion for swimming motility (A), susceptibility to exogenous hydrogen peroxide (B), and twitching motility (C).

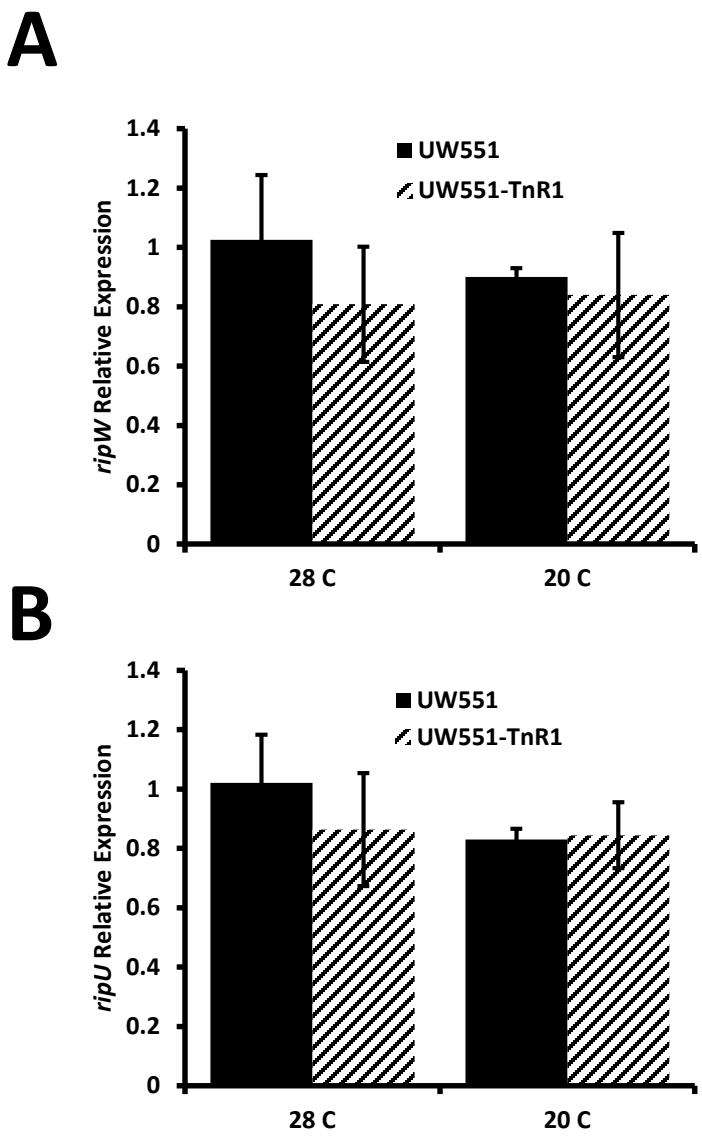


**Figure S2:** qRT-PCR testing of mRNA abundance of the type III effectors *ripW* (A) and *ripU* (B) grown at the indicated temperature in CPG medium shows no difference in expression based on temperature or insertion of transposon in mutant UW551-TnR1.

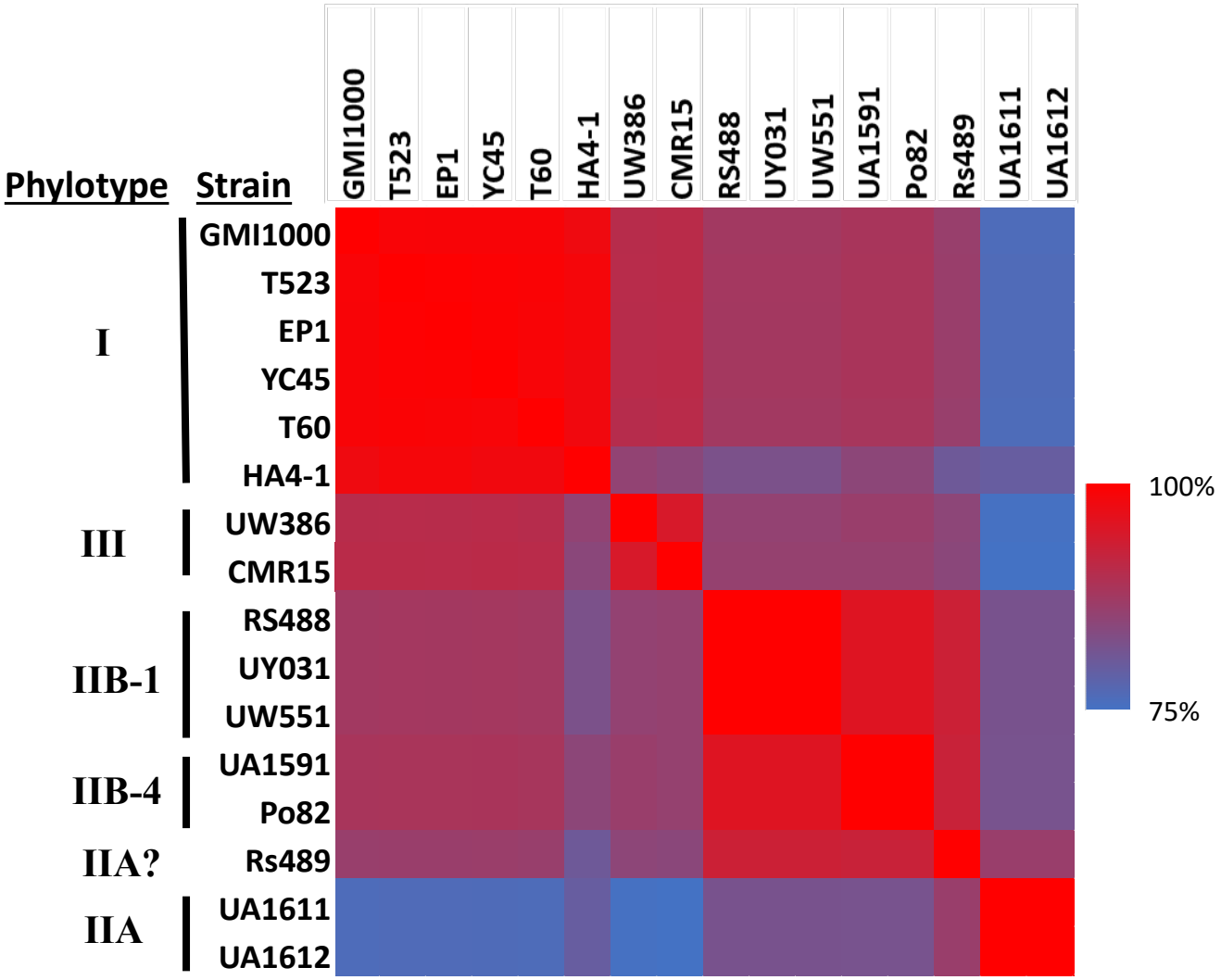


**Figure S3:** Heatmap representing percent identity matrix from multiple sequence alignment of type III effector RipS1 amino acids across strains representing *Ralstonia pseudosolanacearum* and *R. solanacearum* from phylotypes I, IIA, IIB, and III. Cool-virulent phylotype IIB sequevar 1 strains can be separated from other phylogenies based on RipS1 sequence.