Supplementary Materials

Title：RasI/R quorum sensing system controls the virulence of *Ralstonia solanacearum* strain EP1

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Table S3. RT-qPCR validation of the genes differentially expressed in ΔrasI and ΔrasR compared with their parental WT EP1

Gene ID	RNAseq rasI	RT-qPCR rasI	RNAseq rasR	RT-qPCR rasR	Description
AC251_22465	-5.032	-7.052	-1.469	-4.479	*rasI*
AC251_00725	-9.443	-10.396	-8.633	-10.816	fucose-binding lectin II
AC251_00730	-2.591	-4.443	-1.944	-4.703	*solR*
AC251_19280	-2.379	-6.27	-2.176	-6.749	Cellulase
AC251_23955	-1.839	-2.513	-1.862	-2.739	CpaF family protein Type II/IV
AC251_19340	-4.330	-5.136	-4.133	-5.303	SAM-dependent methyltransferase
AC251_17135	-3.204	-2.051	-2.921	-2.228	Universal stress protein
AC251_23985	-2.070	-2.667	-3.253	-3.145	Flp family type IVb pilin
AC251_22035	-2.602	-2.463	-2.779	-3.126	AraC family transcriptional regulator
AC251_25945	-2.374	-3.034	-2.665	-3.583	isopenicillin N synthase family oxygenase

Figure S1

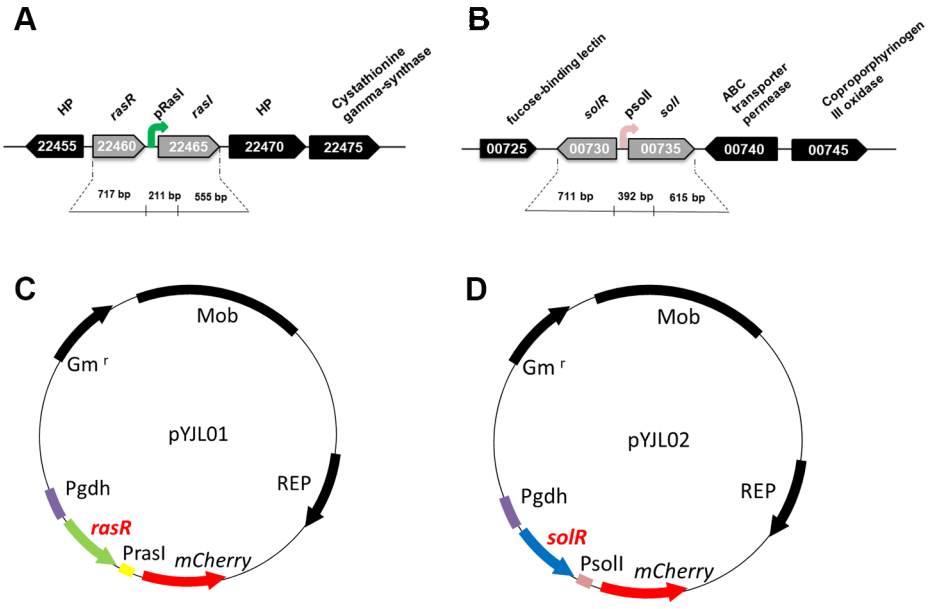

Fig. S1. Construction of reporter strains for AHL QS signals in *R. solanacearum* EP1. (A) The genetic organization of *rasI-rasR* and flanking genes, HP represent hypothetical protein. (B) The genetic organization of *soII-solR* and flanking genes. (C) Genetic map of the reporter construct pYJL01. Pgdh is a strong promoter for driving *rasR* or *solR* gene expression (1). (D) Genetic map of the reporter construct pYJL02.

Figure S2

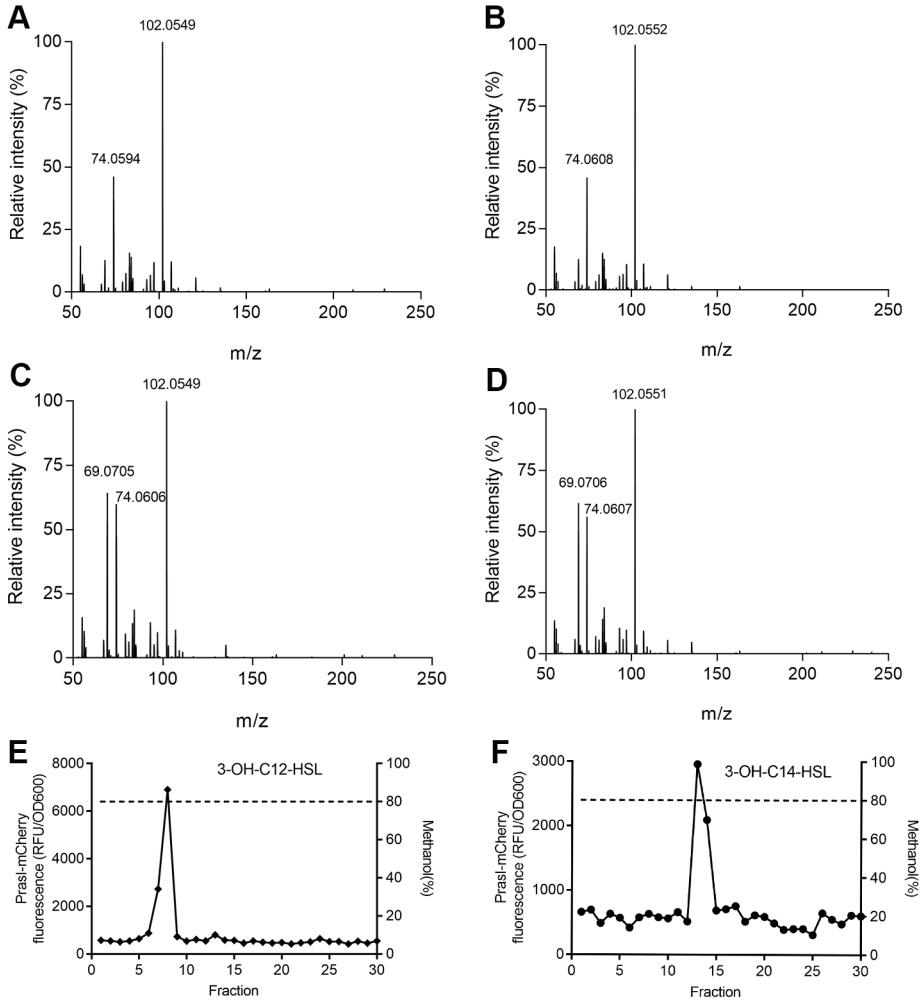

Fig. S2. Purification and characterization of the AHLsignals produced by RasI. MS/MS daughter scan of the m/z 300 (M+H) and m/z 328 (M+H) ion from natural product fractions. (A) and (C), MS/MS daughter ion analysis of the parent ions m/z 300 (M+H) and m/z 328 (M+H) shown in Fig. 2D, respectively. (B) and (D), MS/MS daughter ion analysis of chemically synthesized 3-OH-C12-HSL and 3-OH-C14-HSL, respectively. (E) and (F), Isocratic HPLC profiles of chemically synthesized 3-OH-C12-HSL (◆) and 3-OH-C14-HSL (●), respectively, detected by reporter YJL01. The dashed line shows the methanol level in elution solvent.

Figure S3

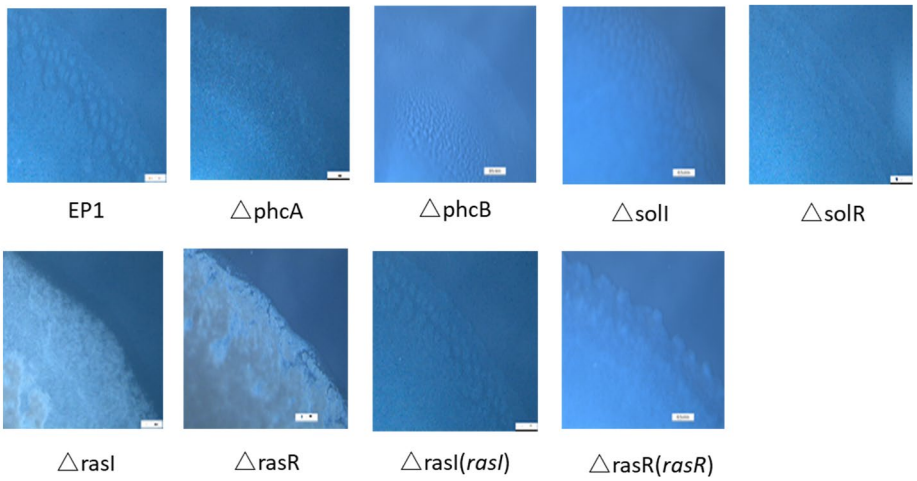

Fig. S3. Morphology of *R. solanacearum* WT EP1 and derivatives grown on twitching motility assay plate. Mutation of *rasI* and *rasR* caused changes in colony morphology and color. Representative images were obtained by using a Leica microsystem (Leica Microscopy, Wetzlar, Germany).

Figure S4

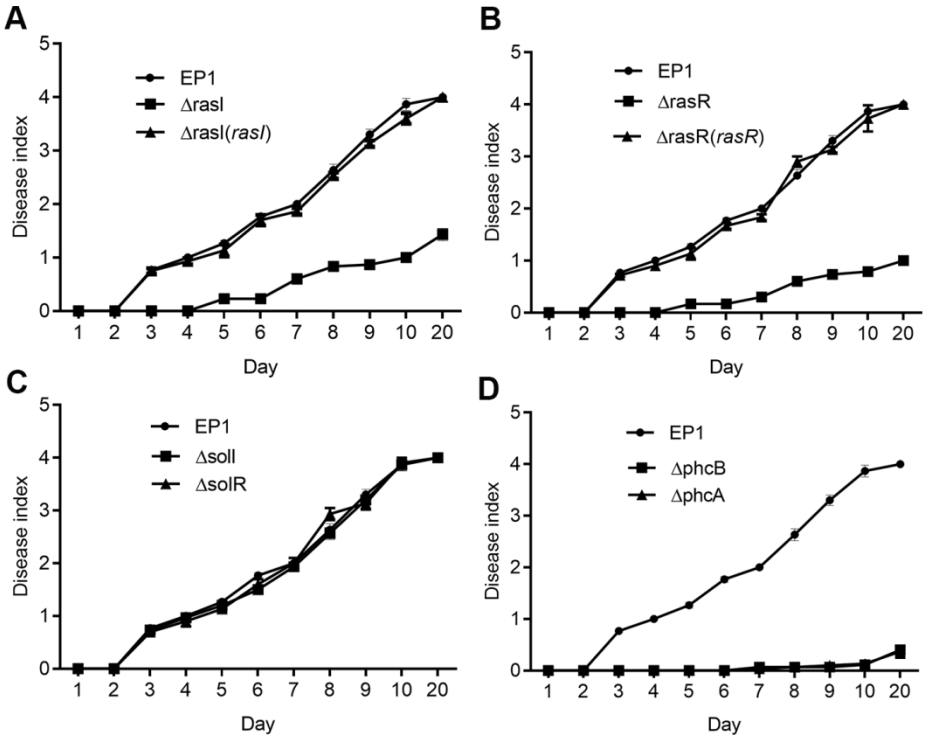

Fig. S4. Virulence assay of *R. solanacearum* EP1 and mutants on eggplant plants. Plants were rated daily on a 0 to 5 disease index scale where 0=healthy and 5=100% died. Data show mean disease index (± SD) for four independent experiments, each containing 10 plants per treatment.

Figure S5

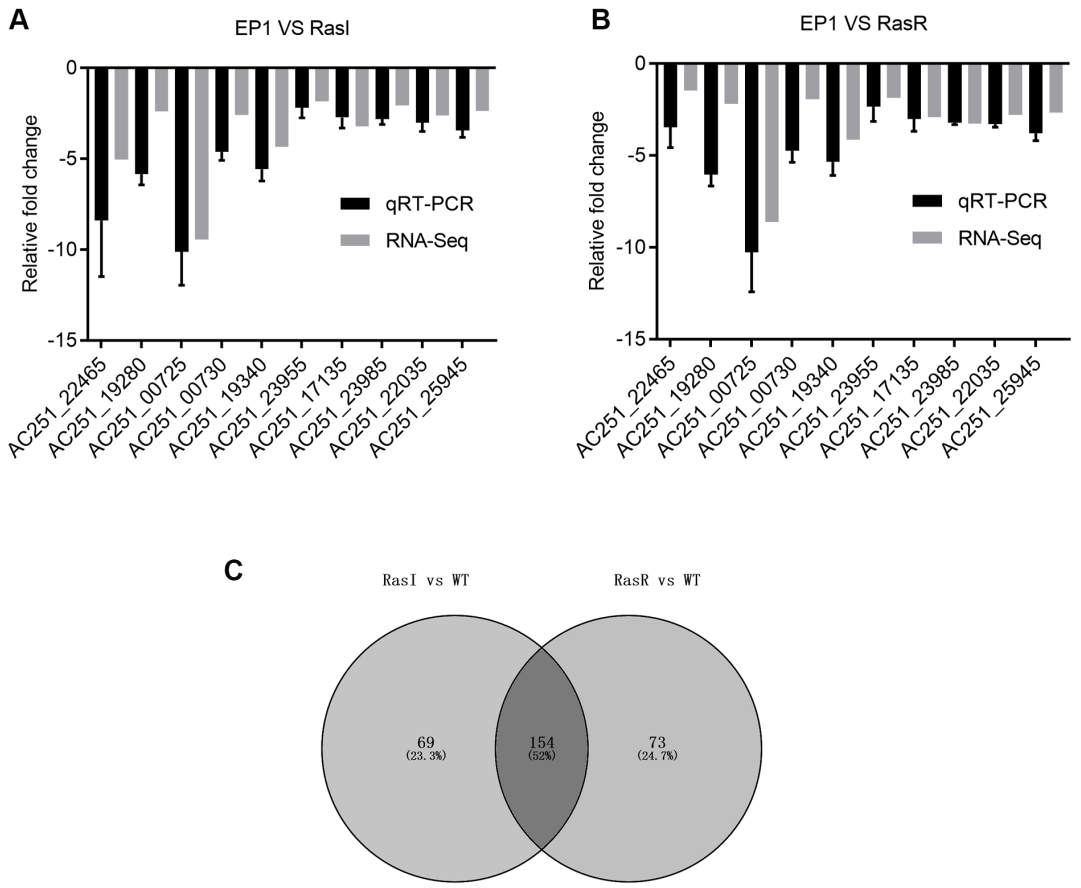

Fig. S5. RasI and RasR regulons in *R. solanacearum* EP1. qRT-PCR validation of RasI (A) and RasR (B) up-regulated and down-regulated genes unveiled by RNAseq analysis (Table 1). qRT-PCR data were normalized to *recA* in WT, ΔrasR, and ΔrasI. Error bars represent SD of three replicates. (C) Venn diagram showing overlapping and specifically regulated genes by RasI and RasR, respectively, in *R. solanacearum* strain EP1.

Figure S6

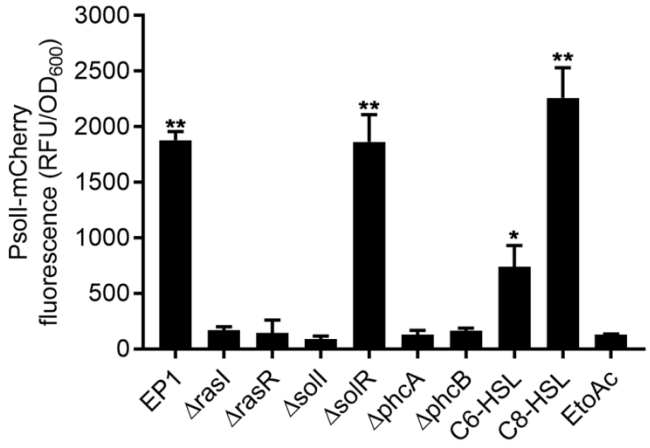

Fig. S6. The *soll* promoter activity in response to the culture extracts from *R. solanacearum* EP1 and mutants. Chemically synthesized C6-HSL and C8-HSL as positive controls. Same volume of ethyl acetate (EtoAC) was added as a negative control. Data are the mean, and error bars indicate the range of three biological replicates. Statistical significance: **, P value < 0.01; *, 0.01<P value < 0.05.

SUPPLEMENTAL LITERATURE CITED

1. **McKinlay, J. B., and C. S. Harwood.** 2010. Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. Proc Natl Acad Sci U S A **107**:11669-75.