**Data in Brief**

**Genome-wide DNA binding pattern of two-component system response regulator RhpR in *Pseudomonas syringae***

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Although *Pseudomonas syringae* uses the two-component system RhpRS to modulate the expression of type III secretion system (T3SS) genes and pathogenicity, the molecular mechanisms and the regulon of RhpRS have yet to be fully demonstrated. We have performed a genome-wide analysis of RhpR binding to DNA prepared from *P. syringae pv. phaseolicola* in order to identify candidate direct targets of RhpR-mediated transcriptional regulation, as described in our recent article [1]. The data are available from NCBI Gene Expression Omnibus (GEO) with the accession number GSE58533. Here we describe the detailed methods and data analyses of our RhpR ChIP-seq dataset.

The bacterial strains used in this study were the *rhpS* mutant from *Pseudomonas syringae pv. phaseolicola* 1448A strain [2] containing either an empty pHM2 plasmid or pHM2-RhpR-HA (C-terminal hemagglutinin-tagged RhpR) [3]. The RhpR expression level in the *rhpS* mutant is 10-fold higher than that in the wild-type strain. These strains were grown at 28 °C in King’s B medium [4] containing the appropriate antibiotics to an optical density at 600 nm (OD600) of 2.0–2.5. The bacteria were centrifuged at 8000 g for 5 min, washed twice with minimal medium (MM, 50 mM KH2PO4, 7.6 mM (NH4)2SO4, 1.7 mM MgCl2, 1.7 mM NaCl and 10 mM fructose, pH 5.7) [5], resuspended in MM to an OD600 of 0.2 and cultured for 6 h at 28 °C before subsequent chromatin immunoprecipitation. Antibiotics (in mg/l) used for the selection of *P. syringae pv. phaseolicola* strains were: rifampicin, 25; kanamycin, 10; and spectinomycin, 50.

**Chromatin immunoprecipitation (ChIP)**

ChIP was performed as previously described [6] with minor changes. After culturing in MM for 6 h, bacteria were treated with 1% formaldehyde (final concentration) for 10 min at 37 °C with shaking. Crosslinking was stopped by adding 125 mM glycine with shaking for additional 5 min. Bacterial pellets were centrifuged at 8000 g for 5 min before washed twice with a Tris buffer (20-mM Tris–HCl pH 7.5, 150 mM NaCl). The pellets were then re-suspended in 500 µl IP buffer (50-mM Heps–KOH pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, mini-protease inhibitor cocktail (Roche)) and sonicated (Fisher 505 Sonic Dismembrator) the DNA to sizes of 100–300 bp (20% total output, 20-second on, 30-
second off, for 2 min on ice). Insoluble cellular debris was removed by centrifugation (12,000 g for 10 min at 4 °C) and the supernatant used as the input sample in IP experiments.

The protein A beads (General Electric) were blocked before added into IP reactions. Protein A beads were resuspended in lysis buffer containing 1 μl of thawed 100× BSA (Bovine serum albumin) for every 100 μl total bead suspension volume, before rotated at 4 °C for 1 h. After spin for 1 min at 12,000 g at 4 °C, the BSA-lysis buffer was removed. The protein A beads were washed in lysis buffer for 3 times on ice. Fifty microliter prepared beads were added into each IP sample (500 μl), and then rotated for 4 h at 4 °C. The mixtures were then incubated by rotating with 50 μl agarose-conjugated anti-HA antibodies (Sigma) in the IP buffer for overnight at 4 °C.

On the next day, the IP reactions were centrifuged and supernatants were removed. One hundred microliter elution buffer 1 (10 mM EDTA, 1% SDS, 50 mM Tris–HCl pH 8.0) was added into each sample and incubated for 10 min at 65 °C. The supernatants were saved after spin. One hundred and fifty microliter elution buffer 2 (0.67% Tris–EDTA buffer) was then added into the remaining beads and incubated for overnight at 65 °C to reverse crosslinking.

On the third day, the elution buffer 1 and elution buffer 2 were combined for each sample. Five microliter 20 mg/ml proteinase K, 10 μl 2 mg/ml glycogen, and 235 μl TE were added into each sample, and incubated for 2 h at 50 °C to degrade all proteins. Fifty microliter 4 M LiCl and 500 μl phenol–chloroform were added into each sample. After spin at 12,000 g at 4 °C for 10 min, the supernatants were collected and precipitated by adding 1 ml cold 100% ethanol. Finally the eluted IP-DNA were centrifuged at 12,000 g at 4 °C for 30 min, and resuspended in 20 μl water. Each IP reaction was performed in duplicate from two separate cultures, which generates two biological replicates.

ChIP-seq library preparation and sequencing

Ten nanogram of co-precipitated DNA was used for ChIP-seq library. DNA fragments (150 to 250 bp) were selected for library construction and sequencing libraries prepared using the NEBflexTM ChIP-Seq Kit (Bioo Scientific). The libraries were sequenced using the HiSeq 2000 system (Illumina) with a 50-bp read length. The resulting sequencing reads (1.35 Gbp including 8.2 M original reads and 3.6 M mapped reads) were over 200-fold coverage of the P. syringae genome (5.93 Mbp in length).

Data analyses

ChIP-seq reads were mapped to the P. syringae pv. phaseolicola A1448 genome using TopHat (version 2.0.0, with the following parameters: -o -solexa-quals -p 3 -bowtie1 -n 2 -g 1) with two mismatches allowed [7]. Only the uniquely mapped reads were kept for the subsequent analyses. The enriched peaks were identified using MACS software (version 2.0.0, with the following parameters: callpeak -t ip.bam -c cbam -f BAM -g 6000000 -nomodel -shiftsize = 75 -n -p 1e-5) [8], which was followed by Multiple EM for Motif Elicitation (MEME) analyses to generate an RhpR-binding motif (with the parameters: -oc -dna -mod zoops -nomotifs 5 -minw 4 -maxw 20 -revcomp) [9]. The top-ranked motif was selected (TGN[T/A][C/ A][N6GATAC][A/G]). P-value = 1.3e-31). More than 80% of peaks are shared in two experiments. The reported peaks are found in both experiments.

Discussion

The TCS RhpRS is a necessary regulator of T3SS in the model plant pathogen P. syringae, and its homologos have been implicated in multiple bacterial pathogens [2]. In order to identify all the in vivo binding sites of RhpR, we have recently presented a genome-wide profile of RhpR binding to chromatin in an rhpS mutant. Our results revealed 167 binding sites including the hrpR promoter, suggesting the direct link between T3SS cascade genes and RhpRS. Most of the peaks contain an inverted repeat (IR)-motif (GTATCN6GATAC). Our data as well as microarray analyses strongly suggest that RhpR is not only a master regulator of T3SS, but also a global regulator of multiple pathways in P. syringae. The ChIP-seq results have provided important information that will help direct the ongoing efforts in determining the regulatory mechanism of RhpR. This work also serves as a framework for further investigations into RhpR functions in P. syringae and other bacteria.

Conflict of interest

The authors state that there are no conflicts of interest.

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