ATAC-Seq Data for Genome-Wide Profiling of Transcription Factor Binding Sites in the Rice False Smut Fungus *Ustilaginoidea virens*

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Abstract

Identification of transcription factor binding sites is one of the most important steps in understanding the function of transcription factors and regulatory networks in organisms. The assay for transposase accessible chromatin sequencing (ATAC-seq) is a simple protocol for detection of open chromatin that could be a powerful tool to advance studies of protein-DNA interactions. Although ATAC-seq has been used in systematic identification of *cis*-regulatory regions in animal and plant genomes, this method has been rarely applied in fungi. Here, we describe a valuable ATAC-seq resource in the genome of an economically important phytopathogen, the rice false smut fungus *Ustilaginoidea virens*. The ATAC-seq data of *U. virens* mycelia collected from potato sucrose broth (PSB) and PSB supplied with rice spikelet extract were both generated. This is the first genome-wide profiling of open chromatin and transcription factor binding sites in *U. virens*.

Transcriptional regulation by a diverse array of transcription factors underpins the control of cellular development and virulence in phytopathogens. Identification of the transcription factors and their cognate transcription factor binding sites is one of the most important steps in understanding the function of transcription factors and regulatory networks. Rice false smut, caused by the pathogenic ascomycete *Ustilaginoidea virens* (teleomorph: *Villosiclava virens*), is one of the most devastating grain diseases in the majority of rice-growing areas of the world (Sun et al. 2020). Rice false smut not only causes yield loss but also threatens human or animal health by producing cyclopeptide mycotoxins in false smut balls (Koiso et al. 1994; Nakamura et al. 1994; Zhou et al. 2012). Genomic analyses of *U. virens* indicated that this fungus might contain over 300 transcription factors (Zhang et al. 2014). However, limited transcription factors have been experimentally verified, including *UvHox2*, *UvCom1*, and *UvPRO1*, which play important roles in development and virulence of this phytopathogen (Chen et al. 2020; Lv et al. 2016; Yu et al. 2019). As such, transcription factor binding sites in *U. virens* in genome-wide scale are not well understood.

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*The e-Xtra logo stands for “electronic extra” and indicates there is supplementary material published online.

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Keywords

ATAC-seq, binding site, transcription factor, *Ustilaginoidea virens*

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Fig. 1. Genome-wide profiling of transcription factor binding sites in *Ustilaginoidea virens* by ATAC-seq. **A**, Distribution of ATAC-seq peaks on the genome of *U. virens*. **B**, Venn diagram showing the overlap of TF-target genes defined by ATAC-seq peaks between the potato sucrose broth (PSB) and rice spikelet extract (RSE) treatments. **C**, Distribution of all ATAC-seq peaks in different regions of the *U. virens* genome. **D**, Logos of position weight coverage matrices from de novo-identified motifs occurring in nucleosome-free ATAC-seq peaks. **E**, Yeast one-hybrid assays showing that transcription factor Uv8b_5800 can bind to promoters of candidate target genes.

Experimental identification methods including DNase footprinting and electrophoretic mobility shift assays have, unfortunately, fallen far behind the rapid accumulation of genome sequences. Moreover, general high-throughput protocols such as ChIP-seq are costly and time-consuming. Recently, ATAC-seq (assay for transposase accessible chromatin sequencing) has been applied to systematically identify *cis*-regulatory regions and DNA footprints in animal and plant genomes (Lu et al. 2017; Wu et al. 2016). Chromatin structure plays a pivotal role in facilitating proper control of gene expression (Boyle et al. 2008; Felsenfeld 1992). Transcription factor binding of *cis* elements is often associated with accessible chromatin regions (Lu et al. 2017). Therefore, the ability to identify these accessible regions throughout a genome will advance the understanding of the relationships among transcription factor binding, chromatin status, and regulation of gene expression. In ATAC-seq, a bacterial transposase, Tn5, that inserts a short fragment of DNA (a transposon) into another molecule of DNA is used (Buenrostro et al. 2013). Since Tn5 is unable to access DNA that is either bound by nucleosomes or has strongly bound transcription factors, it integrates transposons preferentially into accessible or open chromatin (Li et al. 2019). Thus, ATAC-seq can uncover open chromatin regions in which transcription factors might bind. The technology of ATAC-seq has been rarely applied in fungi, such as yeast and mushroom *Sparassis latifolia* (Hendrickson et al. 2018; Yang et al. 2020). However, this technology has not been used in plant-pathogenic fungi.

In this study, we used ATAC-seq to reveal active regulatory regions in *U. virens*. The strain *U. virens* HWD-2, which was isolated from infected spikelets in Wuhan, China (Jia et al. 2015), was used in ATAC-seq experiments. ATAC-seq was performed according to the previously described protocols (Wu et al. 2016). Approximately 0.5 g of mycelia grown in potato sucrose broth (PSB) or mycelia cultured in PSB supplied with 2% (wt/vol) rice spikelet extract (RSE) were collected and were immediately ground in 2 ml of prechilled lysis buffer (15 mM

Table 1. Summary statistics of transcription factor interaction pairs probed by ATAC-seq in *Ustilagoideae virens*

Type of transcription factor ^a	Number of	
	Genes ^b	Interaction pairs ^c
Zn ₂ Cys ₆	73	3,430
Winged helix repressor DNA-binding	39	6,617
Nucleic acid-binding, OB-fold	35	4,945
C ₂ H ₂ zinc finger	32	2,385
Homeodomain-like	20	2,585
HMG	10	1,406
Zinc finger, DHHC-type	10	990
Zinc finger, CCHC-type	8	3,980
bZIP	8	1,047
GATA type zinc finger	6	503
Heteromeric CCAAT factors	6	1,253
Transcription factor jumonji	4	286
bHLH	4	512
Transcription factor TFIIS	4	381
Zinc finger, MIZ-type	3	554
APSES	3	102
MADS-box	2	42
Homeobox	2	126
p53-like transcription factor	2	293
Zinc finger, LSD1-type	2	64
Zinc finger, Rad18-type putative	2	199
AT-rich interaction region	1	195
Lambda repressor-like, DNA-binding	1	69
CCR4-Not complex component, Not1	1	208
RFX DNA-binding domain	1	91
Zinc finger, GRF-type	1	42
Zinc finger, NF-X1-type	1	18
DDT	1	82
YL1 nuclear protein	1	268
Bromodomain transcription factor	1	219
Single-stranded DNA-binding transcriptional regulator	1	160

^a Transcription factor type was classified based on the method of Zhang et al. 2014.

^b Number of transcription factors per family in *U. virens*.

^c Number of transcription factor interaction pairs in *U. virens*.

Tris-HCl, pH 7.5, 20 mM NaCl, 80 mM KCl, 0.5 mM permittine, 5 mM β-mercaptoethanol [2-ME], 0.2% TritonX-100). The total mixture was filtered twice with Miracloth and were then loaded onto the surface of 2 ml of dense sucrose buffer (20 mM Tris-HCl, pH 8.0, 2 mM MgCl₂, 2 mM EDTA, 15 mM 2-ME, 1.7 M sucrose, 0.2% TritonX-100) in a 10-ml Falcon tube. The mixture containing nuclei was centrifuged at 2,200 × *g* at 4°C for 15 min and the pellet was resuspended in 500 μl of prechilled lysis buffer. The nuclei were pelleted by centrifugation at 1,000 × *g* at 4°C for 10 min. The quality of nuclei was checked under microscope by 4',6-diamidino-2-phenylindole staining. Crude nuclei were resuspended in the transposition reaction mix (Mix 25 μl of reaction buffer, 2.5 μl of Nextera Tn5 transposase, and 22.5 μl of nuclease free H₂O) and were incubated at 37°C for 30 min. Qiagen MinElute PCR purification kit was used to purify the DNA after transposition. The DNA was amplified using NEBNext High-Fidelity 2xPCR master mix for 10 to 15 cycles (Lu et al. 2017). Amplified libraries were purified by the Qiagen MinElute PCR purification kit. The library was eluted in 20 μl of elution buffer (10 mM Tris buffer, pH 8.0). The quality of each purified library was assessed using Bioanalyzer and Q-bit. The libraries were multiplexed and were then sequenced using an Illumina HiSeq X Ten platform with 150-bp paired ends by Igenebook Biotechnology Co., Ltd. (Wuhan, China). This experiment was conducted twice.

The adapter and low-quality reads of raw data were filtered out using Trimmomatic (version 0.38) (Bolger et al. 2014), resulting in approximately 28.6 to 49.5 million reads for each treatment. The filtered read data were mapped using Hisat2 software (version 2.0.1) (Kim et al. 2015) to the reference *U. virens* genome (National Center for Biotechnology Information [NCBI] database) (Zhang et al. 2014) with mitochondrial and chloroplast sequences removed. The peak position information on the genome and peak area sequence information

were scanned by MACS (version 2.1.0) (Zhang et al. 2008). A total of 7,089 and 7,081 peaks were identified in the two PSB replicate treatments, while 7,372 and 7,303 peaks were detected in the two RSE replicate treatments (Fig. 1A). The open chromatin ATAC-seq peaks totally targeted 6,443 genes on the *U. virens* genome, and 4,131 genes were common to four independent experiments (Fig. 1B). A total of 299 target genes were found only in the RSE treatments but not in the PSB treatments (Fig. 1B). Of all putative peak regions identified using ATAC-seq, 73.23% of the peaks were located in promoter regions, 13.97% were located in intergenic regions, 9.21% were in coding sequence regions, and 3.59% were in intronic regions (Fig. 1C). We further applied motif discovery to search for candidate transcription factors that could bind these regions. DNA motifs were discovered using multiple expectation maximizations for motif elicitation (MEME, version 5.1.1) (Bailey et al. 2006) of the identified peaks (Fig. 1D). Based on motif sequences, we identified 553,907 transcription factor binding sites, 33,052 interaction pairs, and 285 transcription factors associated with regulation of *U. virens* (Table 1; Supplementary Table S1). We then randomly selected two interaction pairs (Uv8b_5800 and Uv8b_6310; Uv8b_5800 and Uv8b_4095) for yeast one-hybrid verification. The results showed that Uv8b_5800 could interact with Uv8b_6310 or Uv8b_4095 (Fig. 1E), confirming the reliability of the data for predicted interaction pairs. Based on transcription factor binding motifs in the JASPAR database (Sandelin et al. 2004), 23 transcription factors, including six bZIP and 17 homeodomain transcription factors were selected for target-binding site (TBS) prediction in the *U. virens* genome. The TBS prediction analysis revealed that total 7,934 interaction pairs were detected for six bZIP transcription factors, while 4,135 interaction pairs were predicted for 17 homeodomain transcription factors. Compared with ATAC-seq data, TBS prediction showed more interaction pairs and 92.8% of the interaction pairs in TBS prediction analysis were the same as those in ATAC-seq, suggesting that ATAC-seq might provide more precise TBS identification. Hence, the use of ATAC-seq based open chromatin profiling combined with motif discovery provided a valuable resource to identify functional genomic regulatory regions, master regulators, and gene regulatory networks controlling complex in vivo processes in *U. virens*.

All raw and processed data files described in this work were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA643907. The *U. virens* strain HWD-2 was preserved in the China Center for Type Culture Collection (Wuhan, China) under accession number CCTCC M 2011023.

Author-Recommended Internet Resources

JASPAR database: <http://jaspar.genereg.net>

NCBI database: <https://www.ncbi.nlm.nih.gov/nucleotide/JHTR000000000>

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