Journal of Integrative Agriculture 2017, 16(10): 2222–2230

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RESEARCH ARTICLE

Comparative analysis of the genome of the field isolate V86010 of the rice blast fungus *Magnaporthe oryzae* from Philippines

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Abstract

Genome dynamics of pathogenic organisms are driven by plant host and pathogenic organism co-evolution, in which pathogen genomes are used to overcome stresses imposed by hosts with various genetic backgrounds through generation of a range of field isolates. This model also applies to the rice host and its fungal pathogen *Magnaporthe oryzae*. To better understand genetic variation of *M. oryzae* in nature, the field isolate V86010 from the Philippines was sequenced and analyzed. Genome annotation found that the assembled V86010 genome was composed of 1 931 scaffolds with a combined length of 38.9 Mb. The average GC ratio is 51.3% and repetitive elements constitute 5.1% of the genome. A total of 11 857 genes including 616 effector protein genes were predicted using a combined analysis pipeline. All predicted genes and effector protein genes of isolate V86010 distribute on the eight chromosomes when aligned with the assembled genome of isolate 70-15. Effector protein genes are located disproportionately at several chromosomal ends. The Pot2 elements are abundant in V86010. Seven V86010-specific effector proteins were found to suppress programmed cell death induced by BAX in tobacco leaves using an *Agrobacterium*-mediated transient assay. Our results may provide useful information for further study of the molecular and genomic dynamics in the evolution of *M. oryzae* and rice host interactions, and for characterizing novel effectors and *AVR* genes in the rice blast pathogen.

Keywords: *Magnaporthe oryzae*, genetic variation, comparative genomics, effectors, avirulence genes

1. Introduction

Rice (*Oryza sativa*) is the main food for more than half of the world population (Jantasuriyarat *et al*. 2010). Rice blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most destructive diseases of rice growing region of the world (Talbot 2003; Ebbole 2007; Jantasuriyarat *et al*. 2010). Plants have evolved multiple defense mechanisms to protect themselves from infection by a vast range of

Received 28 December, 2016 Accepted 27 March, 2017
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doi: 10.1016/S2095-3119(16)61607-6

microbial pathogens (Jones *et al*. 2006). At present, the effective measures for the control of the rice blast are cultivating resistant varieties and chemical control. Chemical control may not be economic, efficient or environmentally friendly. Further, new resistant varieties can often lose their resistance within a few years of introduction. High genetic variability in the *M*. *oryzae* isolates poses a major challenge to rice breeding and control rice blast disease (Kumar *et al*. 1999). Therefore, control rice blast in rice production remains a problem.

Recent advances in genetic and genomic technology have showed that rice and *M*. *oryzae* emerge as a classical model for studying the plant-microbe interaction (Kim *et al*. 2010). With the rapid development of the sequencing technologies, the genome re-sequencing and comparative analysis studies have been reported in multiple fungal phytopathogens. The genome of the laboratory strain 70-15 of *M*. *oryzae* was the first sequenced among plant pathogenic fungi by using the Sanger sequencing method (Dean *et al*. 2005). Subsequently, field isolates from Japan (Ina168 and P131) and China (Y34) were sequenced by using 454 sequencing technology (Yoshida *et al*. 2009; Xue *et al*. 2012). Additional field isolates from China (FJ81278, HN19311 and 98-06), India (B157, MG01 and RML-29) and the Philippines (R88-002 and Ro1-1) were sequenced by using Illumina high-throughput sequenc ing technology (Ma *et al*. 2010; Chen *et al*. 2013; Dong *et al*. 2015; Wu *et al*. 2015). Interestingly, comparative analysis studies have revealed that they all have their own specific genome regions and hundreds of unique genes. Furthermore, it was observed that there is a high level of variation among the genomes of different isolates (Hu *et al*. 2012) .

High-throughput genome-based studies have provided a new method for cloning novel *AVR* genes. Using genome-wide DNA polymorphisms that exist between field isolate Ina168 and laboratory strain 70-15 of *M*. *oryzae*, Yoshida *et al*. (2009) cloned three novel *AVR* genes, *Avr-Pia*, *Avr-Pii* and *Avr-Pik/km/kp*. Wu *et al*. (2015) identified the AVR effector Avr-pi9 cognate to rice blast gene Pi9 by comparative genomics of requisite strains. More recently, Ray *et al*. (2016) identified a novel *AVR* gene *Avr-Pi54* cognate to rice blast gene *Pi54* from the Indian isolate RML-29 through a comparative genomics study.

Relatively few reports of the genetic variation among different *M*. *oryzae* isolates at genome level have been published. Meanwhile, comparative analysis studies and identification of novel *AVR* effectors will help us to further understand the molecular mechanism of pathogen and host co-evolution. In this study, we performed whole genome sequencing on *M*. *oryzae* field isolate V86010 from Philipines using Illumina high-throughput sequencing technology. Here, we analyzed putative effector protein genes, transposable element (TE) insertion sites and DNA polymorphisms between different strains. Our results reveal the genetic variation characteristic of the different *M*. *oryzae* isolates at the genome level, and to clone novel *AVR* genes from V86010. Functional analysis of these putative effectors will aid in understanding the molecular mechanism of rice-*M*. *oryzae* interaction.

2. Materials and methods

2.1. Rice blast isolates

The *M*. *oryzae* isolate V86010 is kept at International Rice Research Institute (IRRI, Philippines) and 70-15, FJ81278 and Guy11 are kept at Fujian Agriculture and Forest University (FAFU, China).

2.2. Genome sequencing and assembling

The V86010 isolate was cultured at 25°C in complete medium (yeast extract 6 g L^{-1}, casamino acid 6 g L^{-1} and sucrose 10 g L^{-1}) to grow mycelia. And the genome DNA was extracted by using Plant Easy Genomic DNA Isolation Kit from Qiagen (Valencia, CA, USA). The genome was sequenced by using Illumina high-throughput sequencing technology in Beijing Genomics Institute (BGI). We completed V86010 genome assembly using CLC genomic workbench software.

2.3. Gene prediction and annotation

The genomic data were filtered by using Repeatmask3.3 (http://www.repeatmasker.org, species = “magnaporthe_grisea”), using repeat sequences from RepBase (http://www.girinst.org/). Gene prediction used the FGENESH model of Molquest (species, magnaporthe; sequence length, more than 20 aa). We used Repeatmask to predict TEs. Masked genome sequences of V86010 was compared using the MUMMER package to construct the chromosome sequence for V86010 based on reference 70-15 data (Kurtz *et al*. 2004). Secreted protein genes were predicted using SignalP4.1, Protcomp-AN, and TMHMM2.0 (Sonnhammer *et al*. 1998). In this study, proteins were considered to be putative effectors if they were unknown protein which contained N-terminal signal peptide, without transmembrane domain and with less than 400 aa.

2.4. *Agrobacterium*-mediated transient expression in *Nicotiana benthamiana* leaves

Full-length coding sequence (CDS) region with signal peptides for each candidate was amplified and constructed into the plant binary vector pCXSN as described (Chen *et al*.

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