

A Clone Resource of *Magnaporthe oryzae* Effectors That Share Sequence and Structural Similarities Across Host-Specific Lineages

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Abstract

The blast fungus *Magnaporthe oryzae* (syn. *Pyricularia oryzae*) is a destructive plant pathogen that can infect about 50 species of both wild and cultivated grasses, including important crops such as rice and wheat. *M. oryzae* is composed of genetically differentiated lineages that tend to infect specific host genera. To date, most studies of *M. oryzae* effectors have focused on the rice-infecting lineage. We describe a clone resource of 195 effectors of *Magnaporthe* species predicted from all the major host-specific lineages. These clones are freely available as Golden Gate-compatible entry plasmids. Our aim is to provide the community with an open source effector clone library to be used in a variety of functional studies. We hope that this resource will encourage studies of *M. oryzae* effectors on diverse host species.

Resource Announcement

Plant pathogens secrete effectors that play central roles in subjugating plants for colonization. Fungal effectors typically have signal peptides and occasionally carry conserved folds and motifs (Franceschetti et al. 2017; Lo Presti et al. 2015). *Magnaporthe oryzae* (syn. *Pyricularia oryzae*) is an important plant pathogen that is able to infect around 50 species of both wild and cultivated grasses, including important cereals of the Poaceae family. *M. oryzae* is mostly known to cause rice blast but can also cause disease on other crops, such as barley, wheat, foxtail millet, and finger millet. The global population of *M. oryzae* is composed of genetically differentiated lineages that, in some cases, still exhibit a measurable degree of gene flow (Gladioux et al. 2018; Langner et al. 2018). Fungal isolates from each of those lineages show a preference for a specific host and also encode distinct repertoires of effector genes (Yoshida et al. 2016).

The first genomic sequence of *M. oryzae* was released in 2005 for the lab strain 70-15 and allowed researchers to predict a large set of secreted proteins, such as enzymes involved in secondary metabolism, and virulence-associated factors, including putative effectors (Dean et al. 2005). Recently an increasing number of genome sequences of isolates from

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*The *e-Xtra* logo stands for “electronic extra” and indicates that a supplementary figure and a supplementary file are published online.

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different lineages have become available, allowing the research community to perform comparative genomic studies (Chiapello et al. 2015; Yoshida et al. 2016). One of these lineages of *M. oryzae* causes the wheat blast disease and has recently emerged as a serious threat to food security in South East Asia (Islam et al. 2016, 2019). As part of the response to the wheat blast epidemic, we have committed to generating and sharing open source data and resources (Kamoun et al. 2019). This project is part of the wider OpenWheatBlast initiative.

Many of the validated effectors of *M. oryzae* are known as the MAX (*Magnaporthe* avirulence [AVR] and ToxB like) effectors. These effectors, while showing little primary sequence similarity, share a conserved structural fold composed of six β -sheets alternating in an antiparallel manner (de Guillen et al. 2015). The MAX family has expanded in *M. oryzae*, as MAX effectors account for 5 to 10% of the repertoire of secreted proteins and for approximately 50% of the already cloned effectors of *M. oryzae* (de Guillen et al. 2015). Indeed, the identification of structural motifs has enabled more sensitive predictions of effectors from pathogen genomes compared with sequence similarity searches (Franceschetti et al. 2017).

The aim of this project was to computationally identify a set of *M. oryzae* effectors from the main host-specific lineages and to develop an open access clone resource for functional analyses. To develop this resource, we initially analyzed secretomes of 13 *M. oryzae* isolates infecting different hosts, such as rice, wheat, finger millet, foxtail millet, and oat, and of two isolates of *M. grisea*—infecting species of the *Digitaria* genus. The secretomes used in this study have been obtained from the genome resources associated with the studies of Chiapello et al. (2015) and Yoshida et al. (2016). We searched for effector candidates in those secretomes using the following computational pipeline. First, we identified proteins showing sequence similarity to known *M. oryzae* effectors with an AVR activity (i.e., detected by plant immune receptors), using BLAST and a library of 21 previously characterized effectors. Those 21 effectors are AVR-PikD, AVR-Pia and AVR-Pii (Yoshida et al. 2009), AVR1-CO39 (Cesari et al. 2013), AVR-Piz-t (Li et al. 2009), AVR-Pita1 (Orbach et al. 2000), AVR-Pita3 (Khang et al. 2008), AVR-Pib (Zhang et al. 2015), MGG_17227-t26_1 (Zhong et al. 2018), AVR-PI9 (Wu et al. 2015), AVR-Rmg8 (Anh et al. 2018), PWT3 and PWT4 (Inoue et al. 2017), Pwl2 (Sweigard et al. 1995), Pwl1, Pwl3, and Pwl4 (Kang et al. 1995), APikL1, APikL2, APikL3, and ms119 (*unpublished*). The BLAST search was performed with a blosum 62 substitution matrix, a gap opening penalty of 11, and a cut-off E-value of 10. Second, we performed a search for remote relationships using a hidden Markov model (HMM) trained with multiple, structure-based alignments of *M. oryzae* MAX effectors (de Guillen et al. 2015), using the HMMER program (Finn et al. 2011) and the HMM profile generated by de Guillen et al. (2015), with a cut-off E-value of $1e-4$. We performed HMMsearch analyses with a cut-off E-value of 10. Next, we grouped candidates obtained by both approaches to form a nonredundant list of putative effectors and functionally annotated them using two methods. The first is a classical gene ontology (GO) search using the Blast2GO program (Conesa et al. 2005). The second is a BLASTP similarity search against a custom-made database (the Darwin database) that contains more than 2,600,000 predicted proteins of 137 different eukaryotic organisms. We removed candidates with functional domain annotations, as most *M. oryzae* effectors are proteins with no predicted function. Finally, we manually removed candidates with poor gene model predictions and low sequence similarity to *M. oryzae* effectors. The combination of these approaches resulted in 194 effector candidates that we selected for gene synthesis as a clone resource. We also added a homolog of the well-studied *M. oryzae* effector AVR-Pik from the *Lolium perenne* isolate PGKY to this library, bringing the total number to 195 predicted *M. oryzae* effectors.

The Golden Gate cloning system enables rapid and high throughput assembly of multiple sequence modules, such as promotor, terminator, or tags into a common vector (Patron et al. 2015). This cloning strategy is ideal for wide dissemination of cloning material by using a universal code for cloning. We synthesized the 195 *Magnaporthe* species effectors in a Golden Gate-compatible fashion to enable the transfer of these genes into a variety of vectors for various applications, such as yeast two-hybrid assays, heterologous protein expression, and fungal transformation (for example, the fungal transformation vectors described by Pennington et al. [2017, 2018]).

We synthesized the coding sequences corresponding to the mature proteins (without the signal peptide) of the 195 candidates in the Golden Gate-compatible vector pUC57-Kan. Each coding sequence was flanked by *Bsa*I restriction enzyme sites and relevant overhang

Table 1. Features of the 195 *Magnaporthe oryzae* effectors^a

Hosts	Number with similarity to		Total
	AVR effectors	MAX effectors	
Rice	23	27	50
Wheat	6	5	11
<i>Setaria</i> spp.	3	3	6
<i>Eleusine</i> spp.	6	2	8
<i>Avena</i> spp.	3	0	3
<i>Digitaria</i> spp.	20	19	39
<i>Lolium</i> spp.	1	0	1
Multiple hosts ^b	38	39	77
	100	95	195

^a Candidate effectors were selected based on their sequence identity to a known effector of *M. oryzae* or structural similarity with MAX (*Magnaporthe* avirulence [AVR] and ToxB like) effectors according to the hidden Markov model search.

^b Those isolates that infect more than one host among the seven listed hosts.

sequences for Golden Gate cloning reactions. The coding sequences were manually codon-optimized for expression in *Saccharomyces cerevisiae*, *Nicotiana benthamiana*, and *Escherichia coli* (when it was not possible to optimize for all three organisms, priority was given to *S. cerevisiae* and *N. benthamiana*). Table 1 summarizes the different candidates identified, their host specificity, and whether they were selected based on sequence or structural similarities. Supplementary File 1 describes the 195 candidates and includes information such as sequence similarity to *M. oryzae* effectors, results of the HMM search, SignalP scores, functional annotation, and host specificity of the strain in which the effector candidate was identified. An overview of the Golden Gate system and a generic schematic representation of the pUC57-Kan vector insertion site is shown in the Supplementary Figure. The protein sequences and codon-optimized sequences of the selected genes as well as additional information including the plasmid maps of the Level 0 constructs are provided through the data repository Zenodo.

Level 0 modules are available via Addgene. Catalog numbers are provided in Supplementary File S1.

Author-Recommended Internet Resources

Addgene: https://www.addgene.org/Sophien_Kamoun

OpenWheatBlast: <http://wheatblast.net>

Zenodo repository, Kamoun lab Darwin database: <http://doi.org/10.5281/zenodo.3699564>; and for sequences of candidate effectors: <http://doi.org/10.5281/zenodo.3268775>

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