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Review:

Functional genomics in the rice blast fungus to unravel the fungal pathogenicity*

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Abstract: A rapidly growing number of successful genome sequencing projects in plant pathogenic fungi greatly increase the demands for tools and methodologies to study fungal pathogenicity at genomic scale. *Magnaporthe oryzae* is an economically important plant pathogenic fungus whose genome is fully sequenced. Recently we have reported the development and application of functional genomics platform technologies in *M. oryzae*. This model approach would have many practical ramifications in design and implementation of upcoming functional genomics studies of filamentous fungi aimed at understanding fungal pathogenicity.

Key words: Functional genomics, *Magnaporthe oryzae*, Plant pathogenic fungus, Pathogenicity, Rice blast
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INTRODUCTION

The rice blast is one of the most serious diseases on cultivated rice (Ou, 1985). It is capable of destroying enough rice to feed 60 million people every year (Zeigler *et al*., 1994). Since the rice is the staple food and accounts for a significant proportion of caloric and protein intake in many countries (http://www.irri.org/, accessed at 05/08/2008), the disease is one of the major threats to the stable food production worldwide. *Magnaporthe oryzae* (Couch and Kohn, 2002), the causal agent of rice blast disease, has evolved the ability to penetrate and subsequently colonize its host plants. Due to the experimental tractability and socioeconomic impact of rice blast, the fungus has served as an important model organism in the studies aimed at understanding the biology of fungal plant pathogens (Talbot, 2003; Valent, 1990). During the past two decades, infection-specific morphogenesis has been well-defined in the fungus (Dean, 1997; Howard *et al*., 1991; Kankanala *et al*., 2007; Sesma and Osbourn, 2004), and many genes have been identified, which are required for pathogenicity (Talbot, 2003). Nevertheless, it is obvious that our understanding of fungal pathogenicity is still very limited because fungal infection is not a simple phenotype dictated by only dozens of genes but a complicated process that requires the regulated expression and interplay of thousands of genes within the genome.

Recent completion of genome sequencing of *M. oryzae* offered the unprecedented opportunities to examine the genome structure of plant pathogenic fungus and discern possible attributes that confer pathogenicity on the fungus (Dean *et al*., 2005). However, genome sequence information posed new challenges to furthering our understanding of fungal pathogenicity at genomic level. In this review, we discuss these challenges briefly and describe the recent efforts made in our laboratory to address them.

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INSERTIONAL MUTAGENESIS TECHNIQUES IN THE STUDY OF FILAMENTOUS FUNGI

Analysis of genome sequences showed that only a fraction of all genes has assigned biochemical function. In light of this, immediate challenge is to convert the genome sequences into meaningful biological information. This requires the ability to create large mutant collections and to conduct comprehensive screens of resulting mutants. Although systematic deletion of individual genes is the most efficient and straightforward approach to genome-wide screens of gene function, such deletion mutant library has been reported only for the yeast, *Saccharomyces cerevisiae*, as a result of the high efficacy of homologous recombination (Winzeler *et al*., 1999).

Therefore, insertional mutagenesis techniques such as restriction enzyme-mediated integration (REMI) and transposon-arrayed gene knockout (TAGKO) had been developed to generate the mutants and investigate the function of disrupted genes in fungi (Hamer *et al*., 2001; Sánchez *et al*., 1998; Sweigard *et al*., 1998). REMI is one of the most widely used random insertional mutagenesis technique in identifying the genes and their functions important in fungal development and pathogenicity. A big advantage of REMI over the conventional, chemical and radiational mutagenesis technique is that a mutated gene is tagged by the transforming DNA. However, REMI has serious limitations that render it inappropriate for large-scale genomic analysis. REMI protocol requires fungal protoplasts to be prepared, which is time- and labor-consuming. Furthermore, during the transformation process, unsolicited deletion and rearrangement of DNA frequently occur, complicating the analysis of mutant (Mullins and Kang, 2001). Alternatively, *Agrobacterium tumefaciens*-mediated transformation (ATMT), which has long been a workhorse in plant science, has been exploited for fungal transformation. It was demonstrated that *Agrobacterium* is able to deliver its T-DNA into chromosomes of the budding yeast, *S. cerevisiae* (Piers, 1996) and filamentous fungi (Chen *et al*., 2000; de Groot *et al*., 1998). In comparison with REMI, ATMT does not require protoplast and has broad spectrum of starting material to transform. Protoplasts, hypha, spores and even the blocks of mushroom mycelial tissues (Chen *et al*., 2000) could be transformed through ATMT with a higher efficiency than through REMI. During the past half a decade, the number of successful ATMT has increased up to 64 fungal species (Lacroix *et al*., 2006; Michielse *et al*., 2005).

Recently a strain has been developed to have increased frequency of targeted gene replacement (Villalba *et al*., 2008), opening the possibility of large-scale reverse genetics approach in *M. oryzae*. However, as the results indicate, elevated homologous recombination rate might be loci-dependent. In addition, genetic complementation through mating experiment should follow targeted gene replacement using this strain to make sure that no genetic interactions influence the phenotypes of resulting mutants.

ATMT AS A TOOL FOR FUNCTIONAL GENOMICS

We have previously showed that ATMT can be also applicable for efficient insertional mutagenesis in *M. oryzae* (Rho *et al*., 2001). Our laboratory produced a total of 21 070 transformants through ATMT in *M. oryzae* using hygromycin B phosphotransferase as a selectable marker. All the transformants were desiccated and subsequently stored in a plastic box that can accommodate about 1 000 transformants at −70 °C. For a safe maintenance of transformants, one additional copy of library was constructed and deposited in Center for Fungal Genetic Resource (Seoul National University, Korea). A specific ID was assigned to every transformant according to the plate number and coordinates within 24-well plates to which it belongs. Transformant ID allowed systematic management of the library and trackdown of individual transformant from creation and phenotype assay through final analysis of transformants. All the transformants tested were successfully recovered under normal culture condition, and T-DNA insertions in the mutants showed both mitotic and meiotic stability. It was estimated through Southern blot analysis that over 80% of transformants had single T-DNA copy within their genome. In parallel, efforts that have been made in laboratories from USA (Betts *et al*., 2007) and China (Y. Peng, personal communication) produced more than 150 000 ATMT mutants, generating the most extensive insertional mutant

collections in plant pathogenic fungi. If combined together, these mutant resources would be a valuable asset for genetic analysis of *M. oryzae*.

In fungi, however, our knowledge on the pattern of T-DNA integration was limited to the analysis of so small number of T-DNA transformants that the potential of T-DNA as an insertional mutagen in saturating the genome had been uncertain. In an attempt to gain comprehensive view of the T-DNA integration patterns in *M. oryzae*, we analyzed the T-DNA insertion sites and structures in 1246 transgenic strains (Choi *et al*., 2007). For these transformants, thermal asymmetry interlaced-PCR (TAIL-PCR) was employed to rescue border sequences and flanking genomic sequences of T-DNA, leading to the rapid identification of 741 T-DNA-tagged locations (TTLs). Analysis of TTLs showed that T-DNA integration was biased among chromosomes and favored promoter regions of genes having AT-rich base composition. In addition, diverse insertion patterns including direct or inverted repeats of T-DNA, chromosomal rearrangement and readthrough of plasmid vectors were observed. Such diversity in T-DNA insertion patterns had been also reported in plants, but our analysis of junction structures between T-DNA borders and flanking genomic sequences showed that T-DNA integration into fungal genome results in genetic changes such as truncation and deletion less frequently than that into plant counterparts. Similar observations on the patterns of T-DNA distribution and insertion were independently reported by groups in USA and China (Li *et al*., 2007; Meng *et al*., 2007). These results suggest that despite the certain biases, T-DNA insertions are relatively evenly distributed throughout all of the chromosomes and that their canonical insertion patterns may facilitate the genetic analysis of transformants.

HIGH THROUGHPUT PHENOTYPE SCREENING

In the forward genetics approach using random insertional mutagenesis, the next important task for identification of gene function is to assign phenotypes to each mutant. To this end, large-scale nature of functional genomics projects like ours brings two problems at issue. First, phenotypes manifested by genome are so numerous and diverse that detection of mutant phenotypes depends on developmental stages examined and assay conditions employed. Second, application of phenotype assays to the large number of samples is significantly constrained by practical, economic and technical limitations. To overcome these obstacles and facilitate the analysis of functional genes, we developed a high throughput phenotype screening (HTS) system. The HTS system was designed to identify and manage the transformants that are affected in fungal biology and pathogenicity. The HTS system comprises HTS pipeline for phenotype screens and relational database for computerized storage, management and analysis of a vast amount of data from our insertional mutagenesis screens. Design of HTS pipeline was based on the fact that *M. oryzae* can undergo developmental changes away from its host, which allows visual and microscopic inspection of each developmental stage. This, together with the use of 24-well plate format and database, underlies the HTS system. During HTS, all stages of transformants processing and analysis were carried out in 24-well plate format. The use of 24-well plate greatly diminished the time and labor needed to label and handle every transformant because a single plate numbers automatically 24 transformants, which are processed in HTS pipeline as a single unit.

Regeneration of transformants in 24-well plate from the library marks the entry of transformants into HTS system. In our screens of transformants, alterations at seven phenotypes encompassing entire fungal life cycle were monitored in a step-by-step manner. Phenotypes of primary interest were growth rate, pigmentation, conidiation, conidial morphology, conidial germination, appressorium formation, and pathogenicity. In each stage, phenotypes were scored according to the assigned numerical scale. The numerical scale was highly effective in rapid classification of phenotypes, and allowed easy entry of screening results into database. Once transformant cultures in 24-well plate are processed in HTS pipeline, screening data, which are expressed in numerical scale, on seven phenotypes are produced for each transformant and enter database. Monitoring of phenotypes in HTS pipeline is synchronized with transformants cultures in 24-well plate. Pigmentation and vegetative growth rate are checked in 3- and 5-day cultures on V8 medium, respectively. Conidiation, conidial morphology, conidial germination, and ap-

pressorium formation are observed on green mirror, using conidial suspension prepared from 5-day culture. The size of green mirror is fitted to the size of 24-well plate, so that it can hold 24 drops of spore suspension from 24-well plate cultures. Conidial suspensions in 24-well plate are transferred onto green mirror using multi-channel pipette, which speeds up the process. Pathogenicity of transformants was tested on rice plants grown on modified Murashige Skoog media in test tubes. In conventional pathogenicity assay, conidial suspension was spray-inoculated on rice plants grown in pot soil. This protocol requires 3 to 4 weeks of rice cultivation period and green house facility, resulting in time and space constraints in bulk inoculation experiment. Due to high throughput nature of this project, pathogenicity assay was adjusted to 24-well plate format. It took only a week to obtain rice plants suitable for inoculation following our protocol (for more information, refer to the website: http://www.natureprotocols.com/2007/03/20/high_throughput_phenotype_scre.php, accessed at 10/08/2008). All the data produced during the screens entered relational database. Our HTS pipeline enabled a single person to deal with 100 transformants per day, suggesting the application potential of HTS to large-scale mutagenesis program in other filamentous fungi. Through the HPS pipeline, the whole library of 21 070 mutants was processed as a primary screen. An additional round of screen was sequentially carried out to confirm the phenotypes of putative mutants selected from the primary screen. In combination, these screens yielded more than 180 000 data points, which is the most comprehensive phenotypic dataset ever built up for filamentous fungi (Jeon *et al*., 2007).

DEVELOPMENT OF RELATIONAL DATABASE

An enormous amount of data from large-scale phenotype screening and genotyping of mutants obviously lies far beyond the reach of manual information processing and handling. Database is thus required for the information to be housed and managed in a safe and effective fashion to maximize its utility and accessibility. We designed our relational database (ATMT database system) to meet the requirements dictated by experimental protocols in a flexible manner and to implement the interactive analysis between phenotypic and genotypic data (http://atmt.snu.ac.kr/, accessed at 10/08/2008).

ATMT database system consists of two domains: phenotype screening (PS) domain and T-DNA processing (TP) domain. The PS domain archives and retrieves all of the data from HTS to TTLs, connecting over 180 000 data points. The user interface for HTS data registration was designed to have virtual 24-well plate format, so that the data entry into database is simple, and parallel with experimental procedure. This ensures all the data to be entered and handled in an identical manner, reducing introduction of possible human errors. Database users can track down screening records of every transformant using specific ID given to the transformant. Apart from PS domain, TP domain batch-processes T-DNA information. On the receipt of flanking sequences from TAIL-PCR and sequencing, it automatically maps out T-DNA insertion site within the fungal genome, and produces graphical representations of T-DNA insertion. In addition, TP domain provides distribution pattern and density of T-DNA insertions over the whole chromosomes. The automation of tasks relating T-DNA reduced complex processes otherwise required.

In our relational database system, two heterogeneous datasets, phenotypes of mutants and genes tagged by T-DNA, are linked in an interactive way so that contributions of mutation in corresponding genes to phenotypes can be inferred. In light of this, our database system takes large advantage over previously reported databases that have been built for analysis and storage of biological data (Baker *et al*., 2004; Donofrio *et al*., 2005).

SELECTION AND IDENTIFICATION OF PATHOGENICITY LOCI

A total of 21 070 transformants corresponding to 878 plates was processed through HTS pipeline as a primary screen. An additional round of screen was carried out in a sequential way that selected transformants in the primary screen are processed in the secondary. In both primary and secondary screens, transformants of interest were selected with the aid of ATMT database system that provided data retrieval

and grouping analysis. In the primary screen, a total of 2424 transformants were selected from the whole library of 21070 transformants, and in the secondary screen, 559 transformants were finally selected for further characterization.

HTS was designed to assess the phenotypes of transformants by comparing with those of wild type on a qualitative basis. This was appropriate for a large-scale screening scheme. However, qualitative analysis of phenotypes is not sufficient to evaluate the contribution of mutation to phenotypic alterations in corresponding transformant. For an accurate assessment of phenotype, in-depth phenotype analysis was conducted on a quantitative basis for 559 transformants. Basically, the same scheme used in high throughput screening was applied to in-depth phenotype analysis except that the 6-well, instead of 24-well, plate was used and that each phenotype was assessed not only by visual inspection but also by direct, quantitative measurement. The quantitative approach provided phenotype analysis with a higher resolution. A phenotype, which is apparently equal in numerical scale, could be distinguished in in-depth phenotype analysis.

Combined analysis of phenotype and genotype data from our pathogenicity-defective transformants revealed 203 independent loci involved in fungal pathogenicity, presenting the largest, unbiased set of putative pathogenicity genes for a single species. T-DNA insertions in these loci resulted in a broad spectrum of pathogenicity defects ranging from a reduced number and/or size of lesions to no visible symptoms. The pathogenicity genes tagged by T-DNA in our study could be divided into four categories. The first one is *NTH1*, the known pathogenicity gene in *M. oryzae* (Foster *et al*., 2003). The second contains the genes functionally related to the known pathogenicity genes in *M. oryzae.* The third comprises the orthologs of the known pathogenicity genes in other plant pathogens (based on PHI-base search) (Winnenburg *et al*., 2006). The last involves many novel genes and orthologs of genes identified in non-pathogens, accounting for most of genes tagged by T-DNA. Comparison of gene ontology (GO) analysis for pathogenicity genes identified in our study and all genes in *M. oryzae* showed no significant differences between them, indicating the requirement of diverse cellular processes for fungal pathogenicity. To determine whether the T-DNA insertion is linked to mutant phenotypes, we performed targeted disruption for 15 pathogenicity-defective mutants. Linkage was confirmed for 14 mutants, and in only one case, T-DNA insertion does not appear to be responsible for mutant phenotypes (Jeon *et al*., 2007).

SUMMARY AND PERSPECTIVE

Considerable progress has been made during the past decade in elucidating the molecular basis of infection-specific morphogenesis, host penetration, and invasive growth. These advances, however, have not met the growing need for system-level understanding of fungal pathogenesis. Through our work, we have demonstrated how genomics and informatics technologies can be integrated to uncover the genes involved in pathogenicity of *M. oryzae* at genomic scale. We anticipate that our approach will serve as a model for functional genomics in numerous plant pathogenic fungi. Yet one formidable challenge in functional genomics study is to detect as many phenotypes as possible and measure them in a rigorous manner, since no traditional cellular and visual phenotypes were detected in large proportion of mutants. To address this challenge, significant efforts have to be made in developing multi-well plate-based and automated phenotype assays. Collective endeavors of laboratories around the world, when combined with expression profiling, proteomics, and mathematical modeling, would provide the basis of systems biology in *M. oryzae*. System-level understanding of fungal pathogenicity would lead to the paradigm-shift in development of sustainable and environment-friendly control strategy as well as rational breeding strategy.

References

Baker, E.J., Galloway, L., Jackson, B., Schmoyer, D., Snoddy, J., 2004. MuTrack: a genome analysis system for large-scale mutagenesis in the mouse. *BMC Bioinformatics*, **5**(1):11. [doi:10.1186/1471-2105-5-11]

Betts, M.F., Tucker, S.L., Galadima, N., Meng, Y., Patel, G., Li, L., Donofrio, N., Floyd, A., Nolin, S., Brown, D., *et al*., 2007. Development of a high throughput transformation system for insertional mutagenesis in *Magnaporthe oryzae*. *Fungal Genet. Biol.*, **44**(10):1035-1049. [doi:10.1016/j.fgb.2007.05.001]

Chen, X., Stone, M., Schlagnhaufer, C., Romaine, C.P., 2000.

A fruiting body tissue method for efficient *Agrobacterium*-mediated transformation of *Agaricus bisporus*. *Appl. Environ. Microbiol.*, **66**(10):4510-4513. [doi:10.1128/AEM.66.10.4510-4513.2000]

Choi, J., Park, J., Jeon, J., Chi, M.H., Goh, J., Yoo, S.Y., Park, J., Jung, K., Kim, H., Park, S.Y., *et al*., 2007. Genome-wide analysis of T-DNA integration into the chromosomes of *Magnaporthe oryzae*. *Mol. Microbiol.*, **66**(2):371-382. [doi:10.1111/j.1365-2958.2007.05918.x]

Couch, B.C., Kohn, L.M., 2002. A multilocus gene genealogy concordant with host preference indicates segregation of new species, *Magnaporthe oryzae* from *M. grisea*. *Mycologia*, **94**(4):683-693. [doi:10.2307/3761719]

Dean, R.A., 1997. Signal pathways and appressorium morphogenesis. *Annu. Rev. Phytopathol.*, **35**(1):211-234. [doi:10.1146/annurev.phyto.35.1.211]

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H., *et al*., 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, **434**(7036):980-986. [doi:10.1038/nature03449]

de Groot, M.J., Bundock, P., Hooykaas, P.J., Beijersbergen, A.G., 1998. *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. *Nat. Biotechnol.*, **16**(9):839-842. [doi:10.1038/nbt0998-839]

Donofrio, N., Rajagopalon, R., Brown, D., Diener, S., Windham, D., Nolin, S., Floyd, A., Mitchell, T., Galadima, N., Tucker, S., *et al*., 2005. 'PACLIMS': a component LIM system for high-throughput functional genomic analysis. *BMC Bioinformatics*, **6**(1):94. [doi:10.1186/1471-2105-6-94]

Foster, A.J., Jenkinson, J.M., Talbot, N.J., 2003. Trehalose synthesis and metabolism are required at different stages of plant infection by *Magnaporthe grisea*. *Embo. J.*, **22**(2):225-235. [doi:10.1093/emboj/cdg018]

Hamer, L., Adachi, K., Montenegro-Chamorro, M.V., Tanzer, M.M., Mahanty, S.K., Lo, C., Tarpey, R.W., Skalchunes, A.R., Heiniger, R.W., Frank, S.A., *et al*., 2001. Gene discovery and gene function assignment in filamentous fungi. *Proc. Natl. Acad. Sci. USA*, **98**(9):5110-5115. [doi:10.1073/pnas.091094198]

Howard, R.J., Ferrari, M.A., Roach, D.H., Money, N.P., 1991. Penetration of hard substrates by a fungus employing enormous turgor pressures. *Proc. Natl. Acad. Sci. USA*, **88**(24):11281-11284. [doi:10.1073/pnas.88.24.11281]

Jeon, J., Park, S.Y., Chi, M.H., Choi, J., Park, J., Rho, H.S., Kim, S., Goh, J., Yoo, S., Choi, J., *et al*., 2007. Genome-wide functional analysis of pathogenicity genes in the rice blast fungus. *Nat. Genet.*, **39**(4):561-565. [doi:10.1038/ng2002]

Kankanala, P., Czymmek, K., Valent, B., 2007. Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell*, **19**(2):706-724. [doi:10.1105/tpc.106.046300]

Lacroix, B., Tzfira, T., Vainstein, A., Citovsky, V., 2006. A case of promiscuity: *Agrobacterium*'s endless hunt for new partners. *Trends Genet.*, **22**(1):29-37. [doi:10.1016/j.tig.2005.10.004]

Li, G., Zhou, Z., Liu, G., Zheng, F., He, C., 2007. Characterization of T-DNA insertion patterns in the genome of rice blast fungus *Magnaporthe oryzae*. *Curr. Genet.*, **51**(4):233-243. [doi:10.1007/s00294-007-0122-5]

Meng, Y., Patel, G., Heist, M., Betts, M.F., Tucker, S.L., Galadima, N., Donofrio, N.M., Brown, D., Mitchell, T.K., Li, L., *et al*., 2007. A systematic analysis of T-DNA insertion events in *Magnaporthe oryzae*. *Fungal Genet. Biol.*, **44**(10):1050-1064. [doi:10.1016/j.fgb.2007.04.002]

Michielse, C.B., Hooykaas, P.J., van den Hondel, C.A., Ram, A.F., 2005. *Agrobacterium*-mediated transformation as a tool for functional genomics in fungi. *Curr. Genet.*, **48**(1):1-17. [doi:10.1007/s00294-005-0578-0]

Mullins, E.D., Kang, S., 2001. Transformation: a tool for studying fungal pathogens of plants. *Cell Mol. Life Sci.*, **58**(14):2043-2052. [doi:10.1007/PL00000835]

Ou, S.H., 1985. Rice Diseases, 2nd Ed. Commonwealth Mycological Institute, Kew, England.

Piers, K.L., 1996. *Agrobacterium tumefaciens*-mediated transformation of yeast. *Proc. Natl. Acad. Sci. USA*, **93**(4):1613-1618. [doi:10.1073/pnas.93.4.1613]

Rho, H.S., Kang, S., Lee, Y.H., 2001. *Agrobacterium tumefaciens*-mediated transformation of the plant pathogenic fungus, *Magnaporthe grisea*. *Mol. Cells*, **12**:407-411.

Sánchez, O., Navarro, R.E., Aguirre, J., 1998. Increased transformation frequency and tagging of developmental genes in *Aspergillus nidulans* by restriction enzyme-mediated integration (REMI). *Mol. Gen. Genet.*, **258**(1-2):89-94. [doi:10.1007/s004380050710]

Sesma, A., Osbourn, A.E., 2004. The rice leaf blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature*, **431**(7008):582-586. [doi:10.1038/nature02880]

Sweigard, J.A., Carroll, A.M., Farrall, L., Chumley, F.G., Valent, B., 1998. *Magnaporthe grisea* pathogenicity genes obtained through insertional mutagenesis. *Mol. Plant Microbe Interact.*, **11**(5):404-412. [doi:10.1094/MPMI.1998.11.5.404]

Talbot, N.J., 2003. On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. *Annu. Rev. Microbiol.*, **57**(1):177-202. [doi:10.1146/annurev.micro.57.030502.090957]

Valent, B., 1990. Rice blast as a model system for plant pathology. *Phytopathology*, **80**(1):33-36. [doi:10.1094/Phyto-80-33]

Villalba, F., Collemare, J., Landraud, P., Lambou, K., Brozek, V., Cirer, B., Morin, D., Bruel, C., Beffa, R., Lebrun, M.H., 2008. Improved gene targeting in *Magnaporthe grisea* by inactivation of MgKU80 required for non-homologous end joining. *Fungal Genet. Biol.*, **45**(1):68-75. [doi:10.1016/j.fgb.2007.06.006]

Winnenburg, R., Baldwin, T.K., Urban, M., Rawlings, C., Kohler, J., Hammond-Kosack, K.E., 2006. PHI-base: a new database for pathogen host interactions. *Nucleic. Acids Res.*, **34**(90001):D459-D464. [doi:10.1093/nar/gkj047]

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., *et al*., 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science*, **285**(5429):901-906. [doi:10.1126/science.285.5429.901]

Zeigler, R.S., Leong, S.A., Teeng, P.S., 1994. Rice Blast Disease. CAB International, Wallingford.