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Gene Technology

Opinion Article

Recent Technologies Include Transformation Systems, Gene Silencing, and Gene Editing in *Oomycetes*

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DESCRIPTION

Oomycetes are eukaryotic spore-forming microbes that threaten global biodiversity, natural ecosystems by infecting various plant and animal species. It is possible to better understand the infection processes in *oomycetes* and their general biology by using genomics and transcriptomics methodologies in conjunction with host interaction investigations. Some of the molecular mechanisms underlying host invasion and infection by pathogenic *oomycetes* in both plant and animal hosts have been clarified through the use of molecular tools like CRISPR/Cas and RNAi. These techniques provide precise and thorough functional analysis involving numerous areas of research, including genomes, epigenomics, proteomics, and interactomics. To close the information gaps in dynamic biological processes, functional gene characterization is crucial.

There are hundreds of well-known diseases among the varied class of filamentous spore-forming organisms known as *oomycetes*. Several of them are tightly limited by national and international laws and included on worldwide quarantine lists to help stop their spread. Major cultivars of fish and plants, as well as a large number of species found in natural environments, serve as hosts. *Oomycetes* form a taxonomically distinct and large group of eukaryotic microorganisms that shares some physiological and morphological features with fungi but are phylogenetically related to heterokont algae. *Oomycetes* have a wide range of hosts and environmental conditions, which is reflected in their evolutionary diversity. Research on the interactions between hosts and *oomycetes*, along with genomes and transcriptomics has greatly increased our understanding of how *oomycetes* infect their hosts. For the purposeful creation of management strategies, it is critical to understand the role of the numerous interacting molecules. It is known that *oomycetes* secrete a variety of effector proteins that alter the host's immune system to promote infection. For the purposeful creation of management strategies, it is critical to understand the role of the numerous interacting molecules. It is well known that *oomycetes* secrete a variety of effector proteins that alter the immune system of their hosts and make it easier for them to become infected, undergo stable transformation, or use CRISPR/Cas. For *oomycetes*, the development of molecular techniques has advanced more slowly than for fungi; it is now restricted to a small number of species and has low efficiency when compared to fungi. Because oomycetes are heterogeneous, it is necessary to tailor transformation techniques to each species and, frequently, to each strain within each species. Therefore, it is difficult to efficiently convert novel, untested strains.

Pathogenic *oomycetes* in animals

The first animal pathogenic *oomycetes* in which CRISPR/Cas9 has been successfully grown is *Aphanomyces invadans*. In order to alter a target gene in *A. invadans*, zoospores and protoplasts of the pathogen were treated with a guide-RNA paired with Cas9 that forms a ribonucleoprotein (RNP) complex. *A. invadans* is a devastating infection of fish; in particular, carps in Asia are especially sensitive. A serine protease gene was chosen from the genome of *A. invadans* to test the gene mutation system. It was later determined using CRISPR/Cas9 that at least one of the abundantly secreted extracellular proteases from the peptidase S8 domain superfamily contributes to the virulence of A. invadans. Serine proteases have long been thought to be virulence factors in both bacteria and eukaryotes.

In this study, three guide RNAs were created and validated using online prediction techniques for secondary RNA structures and off target sites. A ribonucleoprotein complex was created by combining each of the guide RNAs with Cas9. The individual RNP complexes were subsequently transfected into protoplasts or zoospores utilizing the polyethylene glycol-mediated RNP delivery technique. After amplification, sequencing of the target gene revealed that one of the three RNP complexes utilized frequently caused point mutations in the target gene. The point mutation in the serine protease gene, which is also known to be crucial for the growth and development of many microorganisms, was blamed for the slow growth pattern of the

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transfected protoplasts observed *in vitro* experiments. Stable edited lines were not produced using the CRIPR/Cas9 technique that was designed for *A. invadans*. Theoretically, though, this would be accomplished and would represent a significant advancement in the functional characterization of genes in this and other animal pathogenic *oomycetes*, such *Saprolegnia*.

Gene expression control

Transcription and translation regulate how a gene expresses itself. The quantity and speed of transcription are controlled by the promoter elements that are present upstream of a gene. To start transcription, the RNA polymerase attaches to the core promoter. Therefore, promoter sequences are crucial components of every vector used for functional gene research, regardless of whether it regulates a native gene or a marker gene like green fluorescent protein. Additionally, the Ds Red-Express and hygromycin resistance in *P. nicotine* and *Pythium guiyangense* selectable marker genes, as well as the promoter sequence derived from the glyceraldehyde 3-phosphate gene and the terminator sequence of the *trpC* gene from Aspergillus nidulans, have been used for constitutive expression.