

## CURRENT METHODS OF DETECTION AND IDENTIFICATION OF RICE BACTERIAL PATHOGENS



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Rice is a staple crop for much of the world's population. Two important rice diseases are caused by *Xanthomonas* species. *X. oryzae* pv. *oryzae* (Xoo) colonizes xylem vessels and causes bacterial blight (BB), while *X. oryzae* pv. *oryzicola* (Xoc) colonizes spaces between leaf parenchyma cells to cause bacterial leaf streak (BLS). Both pathogens represent a significant threat for agriculture and global food security, and are considered quarantine organisms in all rice growing countries. With increased production, we observed an emergence of rice bacterial diseases particularly in Africa. Understanding strain diversity, movement, and population dynamics through strain typing is especially important for quarantine pathogens.

Historically, studies were relying on DNA fingerprinting methods such as amplified length fragment polymorphism, restriction fragment length polymorphisms, PCR using rep/box elements, and randomly amplified polymorphic DNA revealed high variability in *Xo* pathogen populations. More recently a multi-locus sequence analysis points to three genetic *Xo* lineages with two sub-lineages: Asian *Xoo*, Asian and African *Xoc*, African *Xoo*, *Xo* isolates from the U.S.A., African and Asian

*X.leersiae*. The recent availability of *Xo* genome sequences has brought this economically important pathogen into the post-genomic era. Indeed, genome sequence availability has led to a technological shift in *Xo* strain typing from fingerprinting approaches to sequence and repeat-based techniques. Complete genome sequences of *Xo* are mined to identify short, hypermutable repetitive elements known as microsatellites or variable number tandem repeats (VNTR). A microsatellite-based typing scheme (MLVA16) has been developed for *Xo* and is useful to highly discriminate among *X. oryzae* strains and to identify the different *Xo* lineages. Other markers specific to additional *Xo* lineages are under development and will further allow to analyze new outbreaks and epidemics of *X. oryzae*.

Molecular diagnostics for crop diseases enhance food security because they enable rapid identification of threatening pathogens and provide critical information for deployment of disease management strategies. Comparative genomics has allowed the identification of regions unique to *Xo*, *Xoo* and *Xoc*. In 2010, these unique regions were used in the design of a multiplex PCR based that is currently used worldwide to diagnose BB and BLS

diseases. A recent advance in molecular diagnostics is the novel loop mediated isothermal amplification (LAMP) method. LAMP allows for rapid, highly specific amplification of target DNA sequences at a single temperature, and is ideal for field-level analysis. We adapted existing genomics-based molecular diagnostic tools for these pathogens into a reliable, sensitive LAMP assay. The specific presence of Xoo and Xoc was detected in DNA,

cells, leaf and seed samples. LAMP for both BB and BLS pathogens will allow surveillance activities in rice fields as well as testing of imported materials by quarantine offices. Genome sequence has also help to identify new species of *Xanthomonas* and to clarify the taxonomic position of strains of *X.oryzae* isolated on weeds. Current works using tools to develop diagnosis and to study the genetic relatedness of *X. oryzae* strains will be discussed.