Synopsis

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is a member of the xanthomonad group of plant pathogens and causes bacterial leaf blight, a serious disease of rice. Like other members of this genus, Xoo has characteristic yellow color due to the presence of the pigment xanthomonadin and mucoidy appearance due to the secretion of extracellular polysaccharide. A number of groups, working in different parts of the world, are using molecular genetic techniques to identify virulence factors of Xoo and other xanthomonads.

An important prerequisite for successful infection is the availability of adequate amounts of nutrients like iron. Under conditions of iron starvation, bacteria secrete low molecular weight iron chelators called siderophores that scavenge iron with high affinity, and make it available to bacteria. Siderophore mediated iron uptake is thus an important factor for survival and growth of bacteria.

Previous studies from our laboratory have revealed that the *rpjF* (regulator of pathogenicity factor; a global regulator of virulence) mutant of Xoo is deficient for virulence and growth on low iron medium. The *in planta* growth deficiency of the *rpjF* mutant can be rescued by supplementation with exogenous iron. Thus functions involved in iron metabolism (uptake, storage, regulation) might be important factors for Xoo virulence. However, the exact mechanisms involved in iron uptake and regulation have not been characterized either in Xoo or other xanthomonads. The objectives of the present study are:

1. Determination of the role of the Ferric uptake regulator (Fur) protein in promoting growth and virulence of Xoo.
2. Identification and characterization of novel Xoo genes involved in iron homeostasis and virulence.

The following sections describe briefly the different chapters of this thesis.

Chapter 1 provides background information regarding virulence functions of xanthomonads. These bacteria employ a diverse array of virulence
factors to gain entry into the host, protect themselves against host defense responses and to procure nutrients from host tissues. Besides functions that are necessary for growth within the host, other xanthomonad functions are likely to play crucial roles in enabling bacteria to survive as epiphytes on plant surfaces prior to host entry. Characterization of these functions would lead to a better understanding of the biology of the xanthomonads and also enable us to design novel strategies to reduce crop losses due to these pathogens.

Characterization of the fur mutant of Xoo is presented in chapter 2 of this thesis. Fur is a master regulator of iron uptake functions in bacteria. A Xoo fur mutant produces siderophores in an unregulated manner, indicating the function of Fur in regulation of iron uptake by Xoo. The Xoo fur mutant grows slowly on normal laboratory medium and exhibits a tendency to revert to fur+. The Xoo fur mutant is also hypersensitive to hydrogen peroxide and has reduced catalase activity. Pathogenicity tests show that the Xoo fur mutant is severely deficient for growth within the host. Growth of the Xoo fur mutant in rice leaves could be restored by supplementation with ascorbic acid, an anti-oxidant. This indicates that the lack of survival of the Xoo fur mutant under in planta conditions might be due to an inability to cope with the oxidative stress conditions that are encountered during infection. Thus Fur appears to have a direct or indirect role in promoting normal growth, iron uptake, detoxification of reactive oxygen species and survival of Xoo within host tissues.

Chapter 3 describes a genome wide screen for the identification of mTn5 induced mutants of Xoo that exhibit altered patterns of siderophore production. About 15,400 mTn5 induced mutants were screened in this study which led to the identification of 35 mutants that exhibit alterations in patterns of siderophore production. Of these, 33 mutants produced siderophore even under iron rich conditions (siderophore over producer mutants; sop mutants) while 2 mutants failed to produce siderophore in iron-starved conditions (siderophore under producer mutants; sup mutants). These mutants represent insertions in 11 genes.
Among the sop mutants, nine had insertions in a pair of genes, colR/colS that code for a putative two component regulatory system. As compared to the wild type strain, Xoo mutants in colR/colS are deficient for virulence and growth in low iron medium. It is of interest to note that although the Xoo genome has more than 25 different two-component sensor and regulator proteins, only mutations in colR/colS were identified in this screen. This indicates an important role for colR/colS in promoting Xoo virulence and growth on low iron medium. Almost one half of the sop mutants carried insertions in a gene (X0O0007), which codes for a conserved hypothetical protein. Mutants in X0O0007 were virulence proficient but unable to grow on low iron medium. Other genes mutated in different sop mutants were metC (X0O3893), acnB (X0O2862), prpB (X0O0892) and prpR (X0O0891), whose products are involved in different metabolic pathways. While mutants in metC and acnB were affected for virulence and growth on low iron medium, those in prpB and prpR were only partially reduced for virulence and unaffected for growth in iron-depleted conditions. Several other genes, like X0O1806, X0O1884 and X0O2024, which code for conserved hypothetical proteins were also identified as carrying mTn5 insertion in the sop mutants. Mutants in the X0O1806 and X0O1884 genes were defective for growth on low-iron medium as well as for virulence. The two sup mutants were virulence proficient but exhibited a deficiency for growth on low iron medium and were mutated in the same gene, X0O0589. This gene has a conserved domain that is present in O-antigen ligases, proteins that function in lipopolysaccharide biosynthesis. Further characterization of these proteins would enable us to understand their role in Xoo pathogenesis and siderophore secretion/production.

This work has aided in identifying a number of genes involved in Xoo virulence that have previously not been reported. The characterization of a Xoo fur mutant and the identification of a putative two component regulatory system as being involved in Xoo virulence are the most significant findings of this work.
Although the *in planta* growth defect of the Xoo *fur* mutant was found to be rescued by ascorbic acid supplementation, the exact mechanism by which Fur promotes Xoo growth and virulence is not known. The identification of CoIR/CoIS implies the presence of a signaling system by which Xoo senses an as yet uncharacterized environmental signal and regulates the expression of a set of genes, which are involved in promoting virulence and growth on low iron medium. Future experiments to identify the Fur and CoIR/CoIS regulons would yield new insights into our understanding of the mechanisms of Xoo pathogenesis. The potential questions that arise from this work and experimental strategies to address these questions are discussed in the “Future plans” section of this thesis.