**SUMMARY**

*Xanthomonas oryzae* pv. *oryzae* is a bacterial pathogen that causes leaf blight, a serious disease of rice. Most members of the genus *Xanthomonas* produce yellow, membrane bound, brominated aryl polyene pigments called xanthomonadins. The functional role of xanthomonadin is not clear. In this thesis, we have conducted a genetic analysis of pigment biosynthesis in *X. oryzae* pv. *oryzae*. The research has been on three broad areas: (1) understanding the function of the pigment xanthomonadin, (2) cloning and characterization of *X. oryzae* pv. *oryzae* genes that are involved in pigment biosynthesis and (3) developing methodology for conducting molecular genetic studies on *X. oryzae* pv. *oryzae*.

Pigment deficient mutants of *X. oryzae* pv. *oryzae* were obtained by both EMS and transposon mutagenesis and these were fully proficient for virulence upon wound inoculation on rice plants suggesting that pigment may not be required for growth of the pathogen within the rice plant. Pigment deficient mutants however showed slight but significant reduction in virulence on epiphytic inoculations. This suggests that the pigment may be required for epiphytic survival of the pathogen on rice leaf surfaces.

Pigment deficient mutants of *X. oryzae* pv. *oryzae* were observed to be hypersensitive to photodamage when compared to the wild type strains indicating that the pigment provides protection against photoinduced damage. A clone containing the genes for xanthomonadin biosynthesis alleviates the hypersensitivity of a pigment deficient mutant. The photoprotective nature of
xanthomonadin is further substantiated by the finding that methanolic extracts containing xanthomonadin reduce the extent of photoinduced lipid peroxidation in liposomes. These results suggest the possibility that pigment might promote epiphytic survival by providing protection against photo oxidation.

A 16.5 Kb region containing genes involved in pigment biosynthesis was cloned from X. oryzae pv. oryzae and reporter (gus) gene fusions to putative pigment promoters were obtained. Marker exchange of these fusions into the wild type X. oryzae pv. oryzae chromosome suggests that the organization of genes at this locus is unique in that genes that are required for pigment production are interspersed with those that may not be required. The phenotypes of marker exchange mutants and complementation analysis suggests that a minimum of five complementation units are present in this gene cluster. These pig::gus transcriptional fusions were used to study the expression of pigment promoters when the pathogen was within the host plant. The range of activities observed with the pig::gus fusions varied from those that were 3-4 times that of the ptac promoter in laboratory medium to some that were less than half the strength of ptac suggesting that the cognate promoters might also have different levels of activity. The pig::gus fusions showed reduced expression in lesion exudates. Likewise, pigment production was also found to be reduced in lesion exudates of the pathogen and this suggests that reduced pigment production maybe due to downregulation of pig gene expression.

Novel pigment deficient mutants that were also affected for the biosynthesis of certain amino acids were identified. Reversion
analysis suggests that single pleiotropic mutations affect both pigment production and amino acid biosynthesis in these strains. The nature of the link between pigment production and amino acid biosynthesis remains to be elucidated. However, there may be common intermediates or regulatory molecules that are required for both pigment and amino acid biosynthesis. The pigment deficient mutant that is also an arginine auxotroph was also virulence deficient. The virulence deficiency in this strain is likely to be due to the arginine auxotrophy as pigment proficient, arginine auxotrophs also revealed a virulence deficient phenotype.

To make these studies possible, I developed methodology for conducting molecular genetic studies on our laboratory strain of *Xanthomonas oryzae* pv. *oryzae*. This included construction and characterization of a cosmid library of *X. oryzae* pv. *oryzae*, transfer of this library into an *E. coli* strain from which clones can be mobilized directly into *X. oryzae* pv. *oryzae*. This mobilizable library has been very useful for other members in the laboratory and clones that complement several interesting mutants have already been isolated. Conditions for mutagenesis of cloned DNA using a mini Tn5gus transposon and marker exchange of these insertions onto the *X. oryzae* pv. *oryzae* chromosome was also standardized. We also obtained evidence for the presence of a strong restriction barrier for introduction of foreign DNA into our laboratory wild type strain of *X. oryzae* pv. *oryzae*, which lacks both of the known restriction-modification systems of this bacterium.
Future Directions

The results obtained so far suggest that pigment deficient strains of *X. oryzae pv. oryzae* are reduced for virulence on epiphytic entry into the host. To further confirm this observation, inoculations can be performed on rice plants in field conditions. Two individual plants, one in each plot, can be inoculated with a pigment proficient and pigment deficient strain of *X. oryzae pv. oryzae* respectively. The spread of bacterial blight of rice can be monitored. A comparison of the number of leaves showing blight symptoms with the pigment proficient strain versus those obtained with the pigment deficient strain would indicate whether pigment deficient strains are reduced for virulence on epiphytic inoculations. For an experiment of this nature, an approval from the DBT and appropriate regulatory authorities would have to be obtained since it involves field release of genetically altered forms of this pathogen.

We also observed that certain *pig::gus* fusions are downregulated when the pathogen is within the rice plant. Not many examples exist in the literature of genes or promoters that are turned down for expression *in planta*. The promoters for genes involved in pigment biosynthesis of *X. oryzae pv. oryzae* can be isolated to determine if they are indeed downregulated.

The organization of the genes in the pigment biosynthetic gene cluster is unusual in that genes that are involved in pigment biosynthesis are interspersed with those that are may not required. Sequence information might provide insights into the nature of the gene products that are encoded by these genes and suggest explanations for this unusual organization. A number of Tn5gus
insertions have been obtained in the xanthomonadin biosynthetic
gene cluster of X. oryzae pv. oryzae. As a beginning, primers that are
outwardly directed from the Tn5 ends can therefore be used to
obtain sequence information.

Novel pigment deficient mutants that are defective in the
biosynthesis of certain amino acids were obtained. Reversion
analysis suggests that a single pleiotropic mutations are
responsible for the phenotypes of these strains. Functional
complementation using clones from the X. oryzae pv. oryzae genomic
library can be conducted to identify the genes that affect both
pigment and amino acid biosynthesis. A clone that restores pigment
production and aromatic amino acid biosynthesis was obtained by
another graduate student in the laboratory (Ajay Goel, unpublished
data) and this confirms that a single mutation affects both pigment
production and the biosynthesis of aromatic amino acids in X. oryzae
pv. oryzae. Likewise complementation of the pigment deficient
arginine and histidine auxotrophs can be conducted to identify clones
that restore both pigment production and prototrophy to these
mutants.

A lot of methodology for molecular genetics of X. oryzae pv.
oryzae was standardized viz. construction and characterization of a
genomic library, conditions for gene transfer into X. oryzae pv.
oryzae and methods for transposon mutagenesis of cloned DNA and
marker exchange of these transposon insertions into the wild type X
oryzae pv. oryzae chromosome. A further advance that would prove
useful are conditions for gene transfer between X. oryzae pv. oryzae
strains using techniques such as genetic transduction, conjugation
etc. Also, the identification of a restriction deficient mutant of the laboratory wild type strain might facilitate gene transfer into this isolate of the pathogen.