Future Plans
FUTURE PLANS

The pathogenic interaction of Xoo with rice provides a good platform for studying plant host factors, bacterial virulence factors, their interplay and the causation of disease. Moreover, the Xoo-rice pathosystem demands a careful study because the consequential disease, bacterial blight of rice, is a devastating disease causing severe yield losses across the rice-growing regions of the world. Several years of research and a lot of recent interest in this field has succeeded in identifying many Xoo virulence factors that are crucial for its pathogenesis (Subramoni et al., 2006; Jha & Sonti, 2009). The work presented in this thesis is a structural and functional characterization of two such virulence factors.

High-resolution crystal structure of the Xoo esterase LipA led to the identification of a unique mode of plant substrate recognition by a bacterial enzyme. This finding has opened several avenues for further exploration. Ongoing work in this direction includes characterization of a natural ligand of LipA from the rice cell wall extracts. Preliminary results show that upon treatment of crude rice cell wall material with LipA, a small soluble molecule ‘elicitor’ is released that can induce defense responses, including programmed cell death, in the rice cells. Presumably, this molecule is a degradation product of the plant cell wall that is sensed by the host upon LipA action. This ‘product’ of LipA action might provide a good estimation of the natural substrate of LipA and a combinatorial approach with NMR, MS-MS and other analytical chemistry techniques can be employed to characterize it. Another interesting aspect of the study can be the crystal structures of LipA homologs from other plant-pathogenic Xanthomonads. Xanthomonas campestris pv. campestris has two LipA homologs while Xylella fastidiosa has three. Since only one of the LipA-like proteins in both species have a carbohydrate-binding pocket, it would be very interesting to identify ‘gain-of-function’ mutation that would endow sugar binding in LipA homologs in these species that do not possess a LipA-like ligand binding. Experiments to alter the sugar specificity in Xoo LipA by site-directed mutagenesis would also be interesting. Finally, the possibility of use of LipA in plant cell wall degrading enzyme cocktails for degradation of agricultural wastes towards biofuel production can also be explored.

Another major aspect of study in this thesis is the expression analysis of the Xanthomonas adhesin-like factor A (XadA) wherein the conditional expression of this virulence factor was
found to be glutamate-dependent and post-transcriptionally regulated. The exciting observation that glutamate, a key component of plant hydathodal exudates, has a role in XadA expression needs to be substantiated with experiments to explore the role of glutamate, if any, in the post-transcriptional control of XadA. The \textit{xadA::gusA} reporter fusion construct generated in this study can be used to perform whole genome screen for regulators of XadA expression, which might reveal a glutamate-sensing regulatory RNA or a completely novel pathway involved in sensing the plant milieu that might also be responsible for the interesting expression profile of XadA. The stable expression of XadA in Xoo is also important knowledge since XadA protein was found to be intrinsically unstable upon overexpression in \textit{E. coli}. The purification strategy of XadA from Xoo and solubilisation of this membrane-anchored protein in SDS can be used for crystallization purposes.

Xoo is under a high selection pressure to survive and efficiently cause disease in the rice host. It is clear from the current study that both LipA and XadA show interesting structural features that confer evolutionary advantage towards proficient entry and infection of the host. Considering the limited available information on structures of Xoo virulence factors, it is essential to generate a repertoire of structures to analyse the adaptive features of the whole Xoo proteome. This would aid in our efforts to understand the Xoo-rice pathosystem intricately, thus adding to the basic knowledge and possibly, to the innovation of new methods of disease control through generation of molecules that block the action of virulence factors or act as novel bactericides.