Chapter 5
Conclusions and future perspectives
5.1 Conclusions and future perspectives

In the current study, we have delineated the role of DSF mediated cell-cell signaling in regulating the virulence of two important rice pathogens, *X. oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*). Using DSF deficient *rpfF* mutant, we have shown that DSF regulation pattern is very atypical amid different strains of *Xanthomonas* spp. This differential regulation pattern for various virulence-associated traits indicate that closely related *Xanthomonas* phytopathogens (*Xoo* and *Xoc*) utilize DSF mediated quorum sensing system to regulate functions, which are specifically required for adaptation and growth to suite particular lifestyle. Using a combined approach of genome wide transcriptional profiling, molecular and biochemical analyses with *Xanthomonas oryzae* pv. *oryzae* strains, we demonstrated that DSF promotes biofilm formation by positively regulating the genes involved in adhesion and EPS synthesis while negatively regulating the motility and chemotaxis associated genes. This regulation is in complete contrast to that reported in *Xcc*, wherein DSF supresses biofilm formation and DSF deficient mutants exhibit aggregated phenotype in broth culture (Dow, 2008; Dow et al., 2003). Our findings indicated that DSF promotes transition from solitary to biofilm lifestyle in *Xoo*, which is required during virulence progression of the pathogen inside xylem vessels. Our results suggested that cell-cell signaling mediated by DSF plays a dual role in promoting colonization at low cell density and establishing infection in the form of biofilm formation during disease process. We have also proposed a model, which provides an understanding about the DSF mediated regulation of different virulence factor, and its significance at various infection stages of bacteria inside host. It will be intriguing to investigate whether and how other important members of *Xanthomonas* fine tunes cell-cell signaling mechanism for effective pathogenesis,
and how the interplay between DSF mediated cell-cell signaling and virulence-associated functions, contribute towards evading host immune responses. Our study further suggested that rpfF mutant overproduces cellular proteins, and oversecretes several Type II effectors. Secretion of extracellular proteins through bacterial membrane mainly depends upon Type II secretion system (Büttner and Bonas, 2010). Although, in our study a Type II secretion single mutant exhibited secretion of negligible amount of protein in extracellular milieu, double mutant with an additional rpfF mutation in T2SS mutant background, displayed significant increase in the protein secretion. Our study indicated that DSF might be regulating certain other protein secretion pathways and/or membrane integrity in Xoo, which needs to be explored in detail. Although it remains to be determined if DSF has any role/s in regulating synthesis of protein or its secretion or both, but sensitivity of rpfF mutant towards membrane damaging agents lend support to the notion that DSF may be contributing towards maintaining the membrane integrity. In addition, detailed analysis of rpfF membrane components compared to wild-type Xoo, and protein secretion studies with membrane damaging and stabilizing agents will help to elucidate the mechanism lying behind the interaction of DSF/RpfF and Type II secretion system.

In addition to DSF regulation in Xoo, we uncovered an important role of ferric uptake system in virulence of X. oryzae pv. oryzicola (a non-vascular pathogen of rice), which is regulated by DSF mediated cell-cell signaling. Through in vitro growth experiments, and transcriptional profiling of DSF deficient ΔrpfF mutant compared to wild-type BXOR1 and complemented strain ΔrpfF (pSC9), we showed that; DSF in Xoc promotes growth under low-iron condition; DSF positively regulates ferric uptake system; DSF deficient ΔrpfF mutants posses low intracellular
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iron, and show defect in ferric (Fe$^{3+}$) iron uptake; DSF positively regulates vibrio ferrin siderophore synthesis, which is required for its virulence; and exogenous iron supplementation promotes in planta growth. Interestingly, our findings revealed that closely related rice pathogens (Xoo and Xoc) employ diverse iron uptake strategies (ferrous vs. ferric) to utilize different forms of iron by modifying DSF mediated signaling to suit their lifestyle within same host. However, a mutant in rpfC and rpfG gene does not phenocopy rpfF mutation in terms of iron related phenotype, although both ΔrpfC and ΔrpfG mutants are deficient in EPS production and virulence inside host, similar to ΔrpfF. In addition, a Δclp mutant in Xoc also behaved differently for these phenotypes. These results have given a perception that signal transduction for DSF dependent regulation of iron phenotype does not occur through RpfC and RpfG two component sensor and response regulator system. An intriguing finding of the current study is an important function of ferric uptake in pathogenesis of Xoc, and future studies will be focused on finding the sensor and regulators for DSF regulated iron dependent phenotypes. In addition, through our global gene expression analysis with Xoc strains, we observed that various two component sensors and response regulators are downregulated in DSF deficient ΔrpfF mutant. In this regard, the future studies can be based on rescuing the iron related phenotype of ΔrpfF mutant by directly expressing these two component sensors and response regulators through plasmid and looking for multicopy suppression. The other way to explore the sensors for DSF regulated iron phenotype can be achieved through reverse genetics approach. Reverse genetics approach employs construction of genomic DNA library in a broad host range vector such as pHM1, and its expression in ΔrpfF mutant background to check for the complementation of the phenotype. The library screening can also provide significant
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clues about various novel components, which might be involved in DSF dependent regulation of different virulence-associated traits in *Xanthomonas*. Identification of novel components and understanding the mechanism of DSF dependent regulation employed by *Xoo* and *Xoc* for pathogenesis could lead to the development of new strategies to reduce the yield loss by these pathogens.

In conclusion, my research findings elucidated the complexity in regulation of virulence-associated functions by DSF mediated cell-cell signaling in different plant pathogens, and indicated that coordination of cell-cell signaling and iron availability plays an important role in regulating the pathogenesis.