# Summary

## Summary

1. Nine putative feruloyl esterase genes (*FAE*) have been identified (*in silico*) in the rice blast fungus *Magnaporthe oryzae* based on protein-sequence homology with the known Fae sequences from *Aspergillus oryzae* (AoFaeB) and *Neurospora crassa* (NcFaeB). These Fae were found to belong to α/β-hydrolase-fold superfamily and showed the presence of a conserved GXSXG motif.

2. Phylogenetic analysis of *M. oryzae* Fae was performed in comparison with 25 other fungal species belonging to different taxonomic classes and orders. The phylogenetic tree showed that Fae sequences had a significant genetic diversity and a discontinuous distribution pattern even among multiple Fae of the same species.

3. Phylogenetic analysis of Fae sequences from different host-specific *M. oryzae* strains, showed that three Fae sequences (i.e., MGG_09404, MGG_09732 and MGG_08737) diverged likely in a host-specific manner.

4. SignalP analysis showed a likely presence of the conventional secretory signal peptide in seven out of nine Fae in *M. oryzae*. Presence of the secretory signal peptide was experimentally validated for two *FAE* genes (i.e., MGG_05529 and MGG 07294) by using Yeast Secretion Trap (YST) vector, confirming the presence of a signal peptide in Fae encoded by genes MGG 05529 and MGG 07294.

5. Effect of host (rice) leaf extract on Fae enzyme activity was checked in vegetative culture of *M. oryzae*. The total Fae enzyme activity in the extracellular (secretory) fraction was found significantly higher (>5-fold) in the presence of host leaf extract, showing that most *M. oryzae* Fae are secretory and that their expression is induced by

the host-derived factors, suggesting a likely role for the CWDE in blast fungal pathogenesis.

6. Transcript levels of nine *FAE*s were studied in response to the individual host cell wall components and also pathogenicity-mimic conditions. Quantitative RT-PCR analysis showed that *FAE* genes did express differentially, with majority of them accumulating >1.5 fold higher in response to the plant cell wall components.

7. The expression of *FAE*s during different stages of infection (*in vivo*) was studied, using barley leaf blades. Remarkably, *FAE1* (MGG_08737), showed a significant increase in relative transcript levels (~300-fold) during pre-invasive appressorial development, host penetration stages (12 and 24 hpi) and also during (~470-fold) the subsequent host colonization stage (48 hpi).

8. *FAE1* gene deletion cassette was made by double-joint PCR approach. *FAE1*-deletion mutant (*fae1*Δ) of WT *M. oryzae* was generated by protoplast transformation to achieve a target replacement of *FAE1* gene with *HPT* gene cassette via homologous recombination. Transformants were screened and confirmed by locus-specific PCR and southern blot hybridization.

9. Although, *fae1*Δ mutant shows normal vegetative growth, asexual conidial and appressorial development, it was impaired in causing blast disease on rice or barley plants in the absence of Fae1 function.

10. Fae1 is required specifically for host colonisation by *M. oryzae*. Invasive growth of the *fae1*Δ mutant was impaired on rice, barley or wheat leaf-sheaths. Genetic complementation of the *fae1*Δ mutant, however rescues its host invasion defect.

11. Exogenous addition of the products of Fae enzyme action, i.e., ferulic acid or glucose, rescues the host invasion defect of the *fae1*Δ mutant, in a dose-dependent manner. However, combinations of these two molecules did not show any remarkable synergistic effect in improving host invasion by the *fae1*Δ. Here, glucose and ferulic acid most likely serves as an energy/nutrient source for the blast fungus.