Chapter 6

Summary

- Bioinformatics analysis was carried out to identify a putative homologue for yeast FAR1 in the rice blast fungus *M. oryzae.* MGG_00134, a hypothetical gene was selected as most probable candidate on the basis of sequence similarity and presence of characteristic domains. This gene was designated as MFAR1.
- The 3.5Kb coding sequence of MFAR1 was amplified and cloned into PYES2 yeast expression vector. The PYES2-MFAR1 plasmid was transformed into yeast Far1 deletion mutant YMP18. It was found MFAR1 can functionally complement the mating defect in yeast Far1 deletion mutant. It was also found that MFAR1 can functionally complement the morphological defects like shmoo formation in yeast Far1 deletion mutant.
- As MFAR1 also shows similarity with yeast scaffold protein Ste5, we investigated whether MFAR1 can functionally complement mating defect and cell cycle arrest defect in yeast ste5 deletion mutant. However, MFAR1 could not functionally complement either of these defects in *S. cerevisiae.* This result suggests that there is no Ste5 like protein in the rice blast fungus.
- Gene deletion of MFAR1 was carried out using split marker gene replacement technology and transformants were confirmed for deletion of gene using gene specific PCR and southern blot hybridization analysis.
- *Δmfar1* mutants were not affected significantly in growth rate but looked morphologically distinct from wild type strain. It was observed that *Δmfar1* mutants produced comparatively less melanin and conidia than wild type strain. However, *Δmfar1* mutants were not affected in appressorium formation and virulence when compared to wild type strain.
- It was observed that *Δmfar1* mutants are affected in female fertility and produced comparatively less perithecia and ascospores than wild type strain. However, male fertility was not affected in *Δmfar1* mutants.

- Subcellular localization showed that MFAR1 is present at low but detectable levels in mycelia and is mainly localized in the nuclear region. It was also found that MFAR1 is expressed at comparatively higher levels in conidia than mycelia.
- One interesting observation was made that yeast α-factor can induce expression of MFAR1 and PMK1 in a MAT1-2 mating type strains but did not significantly affect MFAR1 and PMK1 expression in MAT1-1 mating type strains.
- MFAR1 gene was cloned under the TrpC promoter for strong and constitutive expression of the protein. Two transformants showing maximum expression of MFAR1 were selected for further characterization. Overexpression transformants showed slightly reduced growth rate than wild type strain but most of the morphological features were similar to wild type strain.
- *Δmfar1* mutants in MAT1-2 strains showed comparatively less α-factor mediated inhibition of appressorium indicating that MFAR1 is directly involved in the process.
- Transformants overexpressing MFAR1 showed reduction in appressorium formation and a significant reduction in virulence in comparison to wild type strains. These transformants also showed many conidia forming an elongated germ tube f before appressoria formation. The elongated germ tube phenotype is also shown when the conidia from MAT1-2 type *M. oryzae* strain are treated with lower levels of yeast α-factor.
- Exogenous cyclic AMP restored the appressorium defect in MFAR1 overexpressing transformants, confirming that cAMP overrides the MFAR1 mediated appressorium inhibition in *M. oryzae.* cAMP also restored elongated germ tube formation defect in the overexpression transformants.
- Quantitative real time PCR showed that overexpression of MFAR1 leads to downregulation of MoCyb1, a putative homolog of yeast G1 cyclin Cln2. However, no significant effect was seen on mRNA expression of other cyclins, indicating that MFAR1 specifically regulates expression of MoCyb1.