ABSTRACT

Magnaporthe grisea, the causal agent of rice blast disease, serves as a model system in elucidating the infection pathways of plant pathogenic fungi. With the sequencing of the *M. grisea* genome, there is a wealth of genetic information that needs to be linked to biological function and there is an increased interest to fully understand the various pathways leading to the development of the infection process. *M. grisea* undergoes a series of defined morphogenetic developmental steps, leading to the production of a specialised infection structure called the appressorium, which is essential for infection. Laccases are ubiquitous enzymes in fungi and higher plants and have been shown to play important roles in developmental cycle of various fungi. They are widely distributed oxido-reductases that catalyse the biological oxidation- reduction of polyphenols with a concomitant reduction of molecular oxygen to water. Genomic analysis of *Magnaporthe grisea* using bioinformatics revealed the presence of twelve putative laccases having three multicopper oxidase domains.

In order to characterise these genes at protein level, heterologous expression in yeast and affinity purification were carried out. And to find out the role of these genes in development and differentiation of the fungus, gene silencing studies were carried out in *M. grisea via Agrobacterium tumefaciens*-mediated transformation (ATMT). Silencing vectors were constructed harboring the gene for hygromycin resistance as selectable marker. *M. grisea* transformants were selected on hygromycin (200 µg/ml).

In this study, we characterised two laccases in the fungus *M. grisea,* viz. *MGG_08127.5* and *MGG_02876.5,* which were named as *MoLac1* and *MoLac2,* respectively for our convenience. Bioinformatics analysis predicted that *MoLac1* encodes a protein with three domains of multicopper oxidase with extracellular localisation. Our studies proved that *Mo*Lac1 is a secretory laccase with lignin degadation potential. However,

the knockdown transformants were not affected in growth rate, conidiation and pathogenicity. Taken together, current findings provide evidence that this gene is not essential for the differentiation and development of *M. grisea*.

One of these 12 putative laccases, *MoLac2* was highly induced under nitrogen starvation which mimics pathogenicity conditions. This laccase was also found to be a secretory laccase showing lignin degradation potential, 1, 8-dihydroxynapthalene (DHN) polymerisation ability and ferroxidase activity. The knock-down transformants had weak cell wall and were non-pathogenic. This phenotype was found to be due to silencing of MGG_07771, a cell membrane laccase of *M. grisea*.