INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

SHORT ABSTRACT OF THESIS

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Thesis Title: Investigating the functions of Phospholipase C-1, Ca^{2+}/H^{+} exchanger, and Secretory Phospholipase A_2 in growth, stress responses, and cellulose degradation in *Neurospora crassa*

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SHORT ABSTRACT

Thesis Title: Investigating the functions of Phospholipase C-1, Ca^{2+}/H^{+} exchanger, and Secretory Phospholipase A_2 in growth, stress responses, and cellulose degradation in *Neurospora crassa*

In this thesis work, I investigated the cell functions of three Ca^{2+} signaling genes encoding for a phospholipase C-1 (PLC-1), a Ca^{2+}/H^{+} exchanger (CPE-1), and a secretory phospholipase A_2 ($sPLA_2$) homologs in *N. crassa* using their knockout mutants. The Δ*plc-1*, Δ*cpe-1*, and Δ*splA*$_2$ mutants exhibited reduced survival during induced thermotolerance and increased sensitivity to alkaline pH, but had no effect during cell wall stress in *N. crassa*. Furthermore, deletion of PLC-1 resulted in increased period length and showed loss of temperature compensation at physiological temperature. In addition, the Δ*splA*$_2$ mutant exhibited hypersensitivity in the presence of dithiothreitol (DTT), which induces endoplasmic reticulum (ER) stress, rapid utilization of microcrystalline cellulose compared to the wild type, and increased extracellular protein secretion and glucose accumulation in the culture supernatants. However, when cultured on medium containing sucrose, glucose, xylose, glycerol, and sodium acetate as the

carbon source, the Δ*plc-1*, Δ*cpe-1*, and Δ*splA*$_2$ mutants displayed growth like the wild type. The Δ*splA*$_2$ mutant was unable to grow on microcrystalline cellulose during ER stress. Moreover, studies using the double mutants revealed that *plc-1*, *cpe-1*, and *splA*$_2$ synthetically regulate the circadian clock, stress survival, and utilization of microcrystalline cellulose as a carbon source in *N. crassa*. Furthermore, the homokaryotic transformants expressing the *plc-1*, *cpe-1*, and *splA*$_2$ transgenes under the *ccg-1* promoter complemented the phenotypes of the Δ*plc-1*, Δ*cpe-1*, and Δ*splA*$_2$ mutants during stress conditions and growth on microcrystalline cellulose. qRT-PCR studies revealed differential expression of some important genes involved in regulating the circadian clock, stress responses, and cellulose degradation pathways in the Δ*plc-1*, Δ*cpe-1*, and Δ*splA*$_2$ single and double mutants, implying PLC-1, CPE-1, and sPLA$_2$ crosstalk with these cellular pathways in *N. crassa*. Further, to understand the molecular mechanism of sPLA$_2$ mediated cellulose degradation, I studied the interaction of *splA*$_2$ with *cre-1*, a carbon catabolite repressor. I found that *splA*$_2$ genetically interacts with *cre-1* to regulate vegetative growth and cellulose degradation in *N. crassa*. Further, I predicted the three dimensional protein structures of sPLA$_2$ and CRE-1. Therefore, this study concludes that the *plc-1*, *cpe-1*, and *splA*$_2$ genes are involved in a complex genetic interaction to regulate multiple cellular pathways in *N. crassa*.