Study of cAMP/PKA signaling pathway in *Bipolaris maydis*

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The cAMP/PKA signaling pathway is one of the most important signal transduction pathways. The PKA (cAMP-dependent protein kinase A) plays an important role as downstream effector in this pathway. The PKA was found to be involved in the morphogenesis and virulence in phytopathogenic fungi. Many filamentous fungi possess two genes encoding the catalytic subunits of PKA. However, their specific and redundant functions have not yet been elucidated in *Bipolaris maydis*. In this study, to investigate roles of PKA in this fungus, we characterized two PKA catalytic subunit genes, pka1 and pka2, and generated disruption mutants of these gene, \(\Delta pka1\) and \(\Delta pka2\). \(\Delta pka1\) strains showed severely defected phenotypes in hyphal growth, pathogenicity and sexual development, whereas \(\Delta pka2\) strains showed similar phenotypes as the wild-type. To generate \(\Delta pka1\Delta pka2\) double mutants, we adapted two strategies, sexual hybridization with \(\Delta pka1\) and \(\Delta pka2\), and successive transformation by protoplast-PEG method. By sexual hybridization, an offspring colony of \(\Delta pka1\Delta pka2\) double mutant was not obtained. We carefully studied the genotypes of ascospores in asci using a tetrad analysis technique with a micromanipulator. The ascospores with \(\Delta pka1\Delta pka2\) genotype showed immediate melanization after germination, and stopped hyphal growth within 15 hours. Our observation suggested the possibility that double deletion of *pka1* and *pka2* is lethal. However, by protoplast-PEG method, we obtained \(\Delta pka1\Delta pka2\) candidate strains. The results of their crossing with the wild-type showed occurrence of the suppressing mutation for \(\Delta pka1\Delta pka2\) double mutant lethality in these strains. Currently, we are characterizing the causal mutation by genome comparison.