

## **Cla4 PAK-like kinase regulates asexual/sexual development, pathogenicity and polarity in *Bipolaris maydis***

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PAK (p21-activated protein kinases) -like kinases are master regulators of development and morphogenesis, which are highly conserved among eukaryotes. The broad functions of PAK-like kinases in fungal development and pathogenicity have not been elucidated. In this study, to clarify broad functions of PAK-like kinases in growth, asexual/sexual reproduction and pathogenicity, we identified and characterized two PAK-like kinases, Ste20 and Cla4 in *Bipolaris maydis*, a causal agent of southern corn leaf blight. A single mutant of each *Ste20* or *Cla4* gene was viable, while the double mutant was not available, suggesting the possibility that these genes share essential roles in this fungus.  $\Delta cla4$  strains showed severely defected phenotypes in growth, conidiation, and pathogenicity, while  $\Delta ste20$  strains showed similar phenotypes with the wild-type. Mating tests clarified that Ste20 is dispensable for maternity, while Cla4 is essential for maternal pseudothecium development and also involved in ascospore development in paternal pseudothecium. In summary, Cla4 rather than Ste20 is critical for growth, asexual/sexual reproduction and pathogenicity. Next, in the detail examination of the growth defects in  $\Delta cla4$  strains, we found the alternation of branching pattern in  $\Delta cla4$  strains, elevated frequency of tip splitting. In fluorescent microscopy using FM4-64, temporal loss and subsequent split of vesicle assembly were observed at the hyphal tip in  $\Delta cla4$ , supporting the importance of Cla4 in polarity. Through this study, we clarified conserved functions of Cla4-type in growth, conidiation and pathogenicity among filamentous fungi, and novel functions of Cla4-type in sexual reproduction and polarity.