Genes controlling the infection process of Septoria tritici blotch pathogen of wheat

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Mycosphaerella graminicola (Fuckel) J. Schröt is the causal agent of Septoria tritici leaf blotch, which is the major foliar wheat disease in most temperate areas. Its infection process includes: dimorphic switch from yeast-like form to filamentous growth, penetration of the germtube through stomata, colonization of mesophyll cells, transition from biotrophic phase to necrotrophic phase and finally production of fruiting bodies called pycnidia. In this study we characterized 9 genes belonging to MAP kinase (MAPK) and cAMP pathways including three MAPKs, MgHog1, MgFus3, MgSlt2, the regulatory and catalytic subunit encoding genes of PKA, MgTpk2, MgBcy1, three Ga protein encoding genes, MgGpa1, MgGpa2, MgGpa3, and the Gβ encoding gene MgGpb1. MgHog1 mutants were osmosensitive, highly resistant to the several fungicides and were unable to switch from yeast-like to filamentous growth. MgHog1 mutants were impaired in dimorphic switch, failed to establish infectious germ tubes and therefore were unable to penetrate wheat leaves demonstrating that the dimorphic transition is a key factor in pathogenicity of M. graminicola. Disruption of MgFus3 gene prevented melanization of mycelia and formation of pycnidia in vitro. MgFus3 mutants are non-pathogenic. The mutants of MgFus3 were non-pathogenic, which is ascribed to impaired penetration of stomata, possibly due to inability of the mutants to recognize stomata. In M. graminicola, MgSlt2 plays a role in cell wall integrity since MgSlt2 mutants were affected in polarized growth and showed progressive autolysis during aging. They were also hypersensitive to glucanase and several fungicides and did not produce aerial mycelium or melanin on potato dextrose agar (PDA). The MgSlt2 mutants penetrated wheat stomata regularly, but were unable to establish invasive growth and did not produce asexual fructifications and hence their virulence was severely reduced. Because MgSlt2 is involved in cell wall integrity, MgSlt2 mutants are probably more sensitive to hitherto unknown plant defense compounds, which might explain the compromised colonization of mesophyll tissue. Fructification of M. graminicola is a complex process requiring proper differentiation of the infectious. MgTpk2 and MgBcy1 mutants were able to germinate, penetrate and colonize mesophyll tissue, but were unable to differentiate pycnidia. Our data provide evidence that the cAMP pathway regulates filamentation through MgTpk2 and MgBcy1. Disruption of MgTpk2 impaired filamentation. In addition, the MgTpk2 mutants became melanized faster and secreted a dark-brown pigment into yeast glucose broth medium (YGB), whereas MgBcy1 mutants showed delayed melanization on PDA and were osmosensitive. Overall, the divergent functions of the regulatory and the catalytic subunits of PKA indicate that proper regulation of PKA activity is required for various physiological processes including differentiation, filamentation, osmo-regulation and melanization. MgGpa1 formed fluffy mycelia in liquid medium and hardly produced spores. MgGpb1 mutants showed a nested type of growth on PDA that resulted from hampered filamentation, numerous cell fusions and increased anastomosis. Therefore, we concluded that MgGpa1 negatively regulates filamentation, which is positively regulated by MgGpa3 and MgGpb1. Pathogenicity assays revealed that MgGpa1, MgGpa3 and MgGpb1 are required for virulence of M. graminicola whereas MgGpa2 is dispensable. Based on our results we conclude that M. graminicola is an excellent fungal pathogen model to study molecular mechanisms regulating infection process.