Title Virulence mutants of citrus post harvest disease pathogen *Alternaria citri*

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Abstract

Alternaria citri Elliss & Pierce causes Alternaria black rot, a post harvest fruit disease on a broad range of citrus cultivars. *A. citri* infects either at the stylar or stem ends of citrus fruit and causes internal black discoloration of the fruits core during storage. The other *Alternaria* pathogen of citrus, *A. alternate* rough lemon pathotype, causes brown spot disease on a narrow range of citrus cultivars, e.g., rough lemon and rangpur lime. This pathogen causes necrotic brown spots surrounded by a chlorotic halo on young heaves as results of production of a host-selective toxin, ACR-toxin. The two pathogens are morphologically similar, and the pathogen causing brown spot disease originally was considered to be *A. citri* until its specific host range and production of host-selective toxin were identified. Previously, we proved the role of endopolygalactronase (endoPG) in virulence of *A. citri* or *A. alternata* rough lemon pathotype by gene targeting. The phenotype of the endoPG mutant was significantly reduced in its ability to cause black rot symptoms on citrus. In contrast, an endoPG mutant of *A. alternata* was unchanged in pathogenicity. The results indicate that a cell wall-degrading enzyme can play different roles in the pahogenicity of fungal pathogens. The role of a cell wall-degrading enzyme depends upon the type of disease but not the taxonomy of the fungus. Although endoPG minus mutant of *A. citri* reduced significantly the virulence, the symptom was not completely dismissed and additional factor to endoPG for their pathogenicity was expected. Thus we have initiated a mutational analysis of pathogenicity in *A. citri*, using hygromycin-resistant transformants, generated by restriction enzyme-mediated integration (REMI), and evaluated one of the phenotypes of the REMI mutants of *A. citri*. Pathogenicity-defected strains were selected by REMI, and one of the causes of reduction of pathogenicity was predicted as a histidine auxotroph. Importance of auxotroph on the pathogenicity of pathogenic fungi have been reported in last several years. The *pth*3 mutant of *Magnaporthe grisea* show reduced pathogeniciy and a partial requirement for a histidine (Sweihard et al., 1998). The met1 mutant of M. grisea also showed reduced pathogenicity and a requirement for metionine (Balhadere et al., 1998). *Fusarium oxyporum* f.sp. *melonies* REMI mutagenesis showed hat arginine auxotrophic mutation causes reduced pathogenicity (Namiki et al., 2001). In the case of *A. alternata* apple pathotype, the Arg1 mutant and Arg2 mutant by random mutation showed requirement for arginine and reduced pathogenicity with reduction of their abilities to produce AM-toxin (Tsuge et al., 1987). However, the histidine mutant of the same pathogen showed full pathogenicity against apple leaves (Tsuge et al., 1987). Importance of auxotroph on the pathogenicity is likely different in each fungal pathogen. Thus, a role of histidine auxtotroph in the virulence of *A. citri* was further examined by isolation of gene encoding imidazole glycerol

phosphate dehydratase (IGPD), the sixth enzymes in the histidine biosynthetic pathway, disruption of IGPD gene for causing histidine auxtotroph, and the characteristics of the disruptant including evaluation of the pathogenicity.