Title Elucidation of the biochemical basis of specificity and pathogenicity of *Penicillium digitatum* on citrus fruit

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Abstract

Green mold caused by *Penicillium digitatum* is the most damaging postharvest diseases of citrus fruit. This *Penicillium* species is specific to citrus fruit and do not cause progressive decay in any other fresh fruit or vegetable crops. While the etiology on *P. digitatum* is well understood, the physiological and biochemical basis of its host specificity is much less clear. In this work we report that volatiles emitted from wounded peel tissue play major role in recognition and initial stages of spore germination of *P. digitatum.* Volatiles of various citrus cultivars had a pronounced stimulatory effect on germination and germ tube elongation. When exposed to volatiles from grapefruit, the percentage of germinated spores of *P. digitatum* was 10 fold n as compared to the control. In contrast, *Botrytis cinerea* and *Penicillium expansum* were either not affected or inhibited by the peel volatiles. GS-MS analysis of volatiles present in the peel of various citrus fruit cultivars revealed that limonene is the major fruit peel volatile. Following germination, and before colonization of fruit tissue, *P. digitatum* needs to overcome defense mechanisms in the peel. Our findings suggest that in order to cause decay, *P. digitatum* actively suppresses a defense-related hydrogen peroxide (H_2O_2) burst in citrus fruit. In contrast, inoculation of citrus fruit with a non-pathogenic fungus, *Penicillium expansum,* triggers massive production of H_2O_2 by flavedo tissue. Both fungi induce an elevation in H_2O_2 levels in citrus fruit exocarp from 8 to 17 h after inoculation. Thereafter, *P. digitatum* suppresses H_2O_2 production by host cells and by 66 h the H_2O_2 level was three-fold below that in non-inoculated controls. In wound sites inoculated with *P. expansum*, the level of H_2O_2 was 11-fold above the control value at this time point. Enzymatic removal of H_2O_2 by exogenous catalase, or specific suppression of H_2O_2 production in flavedo tissue by exogenous citric acid, significantly ($P \leq 0.05$) enhanced pathogenicity of *P. digitatum* and even allowed non-pathogenic *P. expansum* to develop lesions on lemon, orange and grapefruit. Theser results, together with recent reports suggesting the potential involvement of citric acid and catalase in green mold pathogenesis, indicate that suppression of the defense-related hydrogen peroxide burst in citrus fruit by these compounds could act as pathogenicity factors for *P. digitatum* on citrus fruit.