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Does increased nutritional carbon availability in fruit and foliar hosts contribute to modulation of pathogen colonization?

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ABSTRACT

High losses due to postharvest pathogens call for an understanding of the processes modulating fungal colonization in comparison to leaf pathogens. Pathogens can penetrate fruit and foliar plants directly through the cuticle following the development of appressoria, or through wounds during fruit growth and postharvest life. In both cases, disease symptoms may not occur until long after infection. Maturation (when plant or fruit has attained maximum growth and size) and ripening (the process by which mature fruit become ready for consumption) are critical factors in fruit susceptibility to postharvest pathogens. During these periods, fruit undergo physiological and metabolic changes that affect nutritional composition and cause a decline in resistance mechanisms. Recent research suggests that host nutrients during ripening may modulate signaling processes in the pathogen that lead to metabolic responses, triggering the accumulation of pH-modulating molecules. The pH shift in the surrounding host tissue induces optimal gene-expression conditions for the fungus to use specific pathogenicity factors at each particular pH. Leaf pathogens usually penetrate via appressoria and also respond to carbon-regulation signaling. This review describes our understanding of the importance of carbon regulation in fruit and leaf tissue, and its contribution to pathogen colonization via facilitation of the transition from quiescent to necrotrophic lifestyle.

1. Introduction

The susceptibility of harvested fruit to pathogen colonization is a dynamic process that is regulated by the fruit during its maturation and ripening. Disease symptoms of postharvest pathogens usually occur at later periods after fruit infection. The period between fruit infection and symptom development is called the quiescent stage (Prusky et al., 2013). During ripening of the host, the quiescent infections are activated, showing decay symptoms and modulating the host physiology (Prusky, 1996).

The biotrophic interaction with postharvest pathogens involves initial penetration of the germinated hypha into different tissues of fruit and vegetables (fruit peel, receptacle, flower stems, etc.) without any specific fungal structure that might facilitate the intimate parasitic interaction. The complete absence of haustoria from the postharvest pathogen's life cycle suggests that the germinated spore has no biotrophic stage and probably enters immediately into necrotrophic development (Prusky et al., 2013). However, in hemibiotrophs such as *Colletotrichum*, the fungus has an initial biotrophic interaction, followed by activation of a quiescent stage that culminates in a necrotrophic stage with disease symptoms (Prusky et al., 2013).

Early work suggested that four factors modulate termination of the quiescent interaction: (i) induced accessibility of cell wall substrates during ethylene evolution and softening of the climacteric fruit (Cantu et al., 2009); (ii) reduced concentrations of preformed antifungal compounds and inducible phytoalexins; (iii) a decline in host-defense responses (Beno-Moualem and Prusky, 2000); (iv) conducive pH and carbon availability in the ripening host (Prusky et al., 2016).

The termination of quiescent infections is then the result of the fungus's ability to initiate attack under the dynamic physiological conditions developing during host maturation. Fruit ripening is accompanied by significant metabolic changes that affect fruit resistance, as well changes in the host (sugar accumulation, changing pH) contributing to the activation of pathogenicity factors (Prusky, 1996). During this period, the pathogen must contribute to colonization by metabolizing host tissues, thereby making the nutrients required to sustain its growth available. This suggests that conditions imposed by the host during fruit ripening or leaf development contribute to its

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susceptibility. In this review, we focus on the possibility of host sugar metabolism contributing to fungal pathogenicity.

2. Sugar metabolism in ripening fruit

Carbohydrates provide energy and building blocks for plant growth and development. In addition, sugars may act as signal molecules, regulating the expression of many key genes involved in plant metabolic processes. Plants have developed a very efficient system for sugar metabolism and accumulation in sink cells termed sucrose–sucrose cycle (Li et al., 2012). When sugars are transported to sink cells (e.g., fruit, roots or shoot tips), they are converted to fructose and glucose. Most of the sucrose, glucose and fructose, and other soluble sugars that have not been metabolized, are transported into the vacuole. Once inside the vacuole, sucrose can also be converted to glucose and fructose by vacuolar acid invertase. Thus, the plant modulates sugar metabolism toward its accumulation in subcellular compartments.

The concentration and distribution of sugars in plant cells are modulated by the sucrose–sucrose cycle system, which is affected by developmental processes in each tissue type and environmental conditions (Dai et al., 2011). Sucrose accumulation in ripening melon (*Cucumis melo*) fruit is attributed to higher sucrose phosphate synthase activity and lower invertase activity compared to young fruit (Dai et al., 2011). The expression levels of rice (*Oryza sativa*) hexokinases (HKs) *OsHK5* and *OsHK6*, which function as glucose sensors, are upregulated in rice leaves by exogenous application of glucose and fructose (Cho et al., 2009). In apple fruit, at the early stage of fruit development, imported sugars are used mainly for cell division and growth. During fruit maturation starch breaks down, followed by continued sucrose accumulation in the vacuole. The sugars released from starch are ultimately glucose, fructose, and sucrose, which are used for respiration and to enhance the sweetness of the ripening fruit; at the same time, they are acquired by fungi and contribute to pathogenicity.

3. Nutrient acquisition in fungi

Plant-pathogenic fungi acquire and use nutrients during infection. Sugar availability during fruit maturation and ripening is important, given that nutritional factors modulate the use of fungal regulatory pathways. Those pathways may activate the secretion of enzymes to macerate cell tissue and release substrates for fungal consumption. Fungal global carbon and nitrogen regulators ensure optimal use of glucose and ammonium first, before the less favored alternatives, which are repressed. The control of nutrient acquisition by fungi on complex substrates is well regulated and pathogens must respond to the dynamic changes in carbon and nitrogen sources as a tool to improve pathogenicity.

How, then, does the metabolic regulation by fungi occur during the transformation from quiescent to active infections? When and how do the fungal systems switch from their own stored nutritional factors to those induced in the host? What are the signals that activate fungal primary metabolism to produce the initial molecules contributing to fungal pathogenicity? What differential metabolism is activated to acquire nutrients from the immature developing host tissue vs. the fully mature one? What are the metabolic strategies contributing to pathogen maceration and colonization? Transcriptional, biochemical, and functional analyses of fungal genes in biotrophic and hemibiotrophic foliar pathogens and necrotrophic fruit pathogens have attempted to answer these questions, and to characterize the contribution of fungal metabolism during plant infection.

The transduction pathways induced by specific nutrients bring about changes in gene expression, mRNA stability (Cereghino and Scheffler, 1996) and post-translational modifications (Ordiz et al., 1996). Although several of the genes implicated in the pathways that control glucose repression and induction have been identified (Gancedo, 1998), a complete mechanistic picture of the phenomenon is not yet available for foliar or fruit pathogenic fungi. In particular, the position of each factor in the signaling cascade and the interactions among them are still not well known.

The importance of fungal HKs and their activation in pathogenic fungi have not been fully described. What differentiates HK 2 in yeast from other HKs is that not only is it the enzyme responsible for the phosphorylation of glucose, but it is also implicated in glucose repression, indicating the complexity of carbon metabolism at initial stages of fungal colonization (Moreno and Herrero, 2002).

4. Fungal feeding properties

Postharvest pathogens with different lifestyles may exploit various host-derived nutrients. Necrotrophs, including *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Penicillium expansum*, kill the host to acquire nutrients (van Kan, 2006). Hemibiotrophs, including *Colletotrichum* spp. (Munch et al., 2008) and the rice blast fungus *Magnaporthe oryzae* (Yi and Valent, 2013), may show a series of activated transporters for nutrient uptake. The importance of nutrition, once the appressoria have breached the fruit or leaf cuticle, is not well known. True biotrophs that are not postharvest pathogens develop haustoria as a specialized feeding structure to acquire nutrients from cells (Voegele and Mendgen, 2011). Interestingly, in those cases, the expression of genes involved in primary metabolism, as well as those encoding carbohydrate-active enzymes (CAZys) in obligate biotrophs, is reduced relative to non-biotrophs, suggesting that they are involved in primary metabolism.

In contrast to biotrophic stages of development, necrotrophs have no specialized feeding structure: instead, they secrete enzymes that degrade the plant cell wall and membrane (van Kan, 2006). *B. cinerea* and *S. sclerotiorum* genomes carry genes for nitrate assimilation and multiple plant cell wall-degrading (CAZys) enzymes. The genomes of the necrotroph *P. expansum* and the hemibiotroph *Colletotrichum* spp. are both enriched for CAZy genes (Alkan et al., 2015; Barad et al., 2016), enzymes that are expressed during maceration, in a glucose-dependent manner (Fernandez et al., 2012). CAZy expression in *Colletotrichum* attacking fruit is probably controlled by environmental pH changes, which are linked to nutritional changes. A wide occurrence of CAZys was also observed in the different P. expansum clusters, where 771 putative CAZys were identified (Barad et al., 2017). In both cases it is clear their importance in plant cell-wall degradation and their contribution to colonization of the host fruit. Despite the differences in their attack mechanisms, all pathogens must, at some point, acquire and assimilate carbon from the host.

5. Nutritional conditions that determine the secretion of small molecules modulating pathogenicity

Fungal pathogens respond to nutrient fluctuations in ripening fruit or growing plants by modulating the host's environmental pH (Prusky et al., 2016). Through this mechanism, changing sugar concentrations during fruit maturation may affect pathogenicity. This mechanism is regulated by carbon catabolite repression (CCR), which ensures the utilization of glucose over that of less favored nutrients. Differential carbon levels as sucrose or glucose may affect metabolic processes differently, resulting in varying biochemical outcomes. Limiting carbon availability at the leading edge of the colonized tissue in ripening fruit induces accumulation of the weak base, ammonia, by *Colletotrichum gloeosporioides*. Under the high sucrose concentrations present in ripe fruit, fungal pathogens may oxidize sugars, inducing the production of organic acids and decreasing local host tissue pH. While wound-penetrating necrotrophic pathogens are usually directly affected by nutritional regulation and pH response, biotrophic pathogens that renew their development will remain quiescent until suitable host sugars are available. These will eventually activate a fungal HK or HK-like protein that may be involved in regulatory pathways triggering CCR. In both cases, the fungus will produce ammonia as an initial pathogenicity