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REVIEW

Organic acid, a virulence factor for pathogenic fungi, causing postharvest decay in fruits

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Abstract

Decay due to fungal infection is a major cause of postharvest losses in fruits. Acidic fungi may enhance their virulence by locally reducing the pH of the host. Several devastating postharvest fungi, such as *Penicillium* spp., *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, can secrete gluconic acid, oxalic acid, or citric acid. Emerging evidence suggests that organic acids secreted by acidic fungi are important virulence factors. In this review, we summarized the research progress on the biosynthesis of organic acids, the role of the pH signalling transcription factor PacC in regulating organic acid, and the action mechanism of the main organic acid secreted via postharvest pathogenic fungi during infection of host tissues. This paper systematically demonstrates the relationships between tissue acidification and postharvest fungal pathogenicity, which will motivate the study of host–pathogen interactions and provide a better understanding of virulence mechanisms of the pathogens so as to design new technical strategies to prevent postharvest diseases.

KEYWORDS

acidic fungi, ambient pH, organic acids, regulatory mechanism, virulence factor

1 | INTRODUCTION

Decay caused by fungal infection is the most extensive loss in postharvest fruits, and unsuitable postharvest ambient conditions may accelerate the onset of infection during storage and transport of fruits. Pectate lyase has been implicated as an essential virulence factor, and pectin compounds in the pectinolytic intermediate layer can cause impregnation and softening of host tissues. However, not all cell wall-degrading enzymes produced by pathogenic pathogens are necessarily virulence factors (Miyara et al., 2008). Fungal pathogens can enhance their infecting ability by secreting organic acids or ammonia to acidify or alkalize the host's environment. Based on these mechanisms, fungal pathogens are classified into two categories, acidic and alkaline fungi (Prusky & Lichter, 2008). *Penicillium digitatum*, *Penicillium italicum*, *Penicillium expansum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and other postharvest fungi have been identified as acidic fungi, causing green mould in citrus (Ramón-Carbonell & Sánchez-Torres, 2017; Smilanick et al., 2005), blue mould in apple and peach, and grey mould in tomato, strawberry, and grape (Jiao et al., 2018; Levine et al., 1994; Ugolini et al., 2014). Secretion of

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organic acids is important during invasion and pathogenesis of such fungi, as the environmental pH effectively regulates fungal growth and development as well as increasing fungal pathogenicity (Prusky et al., 2001). Bateman and Beer (1965) reported a close association between fungal pathogenicity and pH value. They proposed that pathogens acidify the host tissues during infection and produce pH conditions more suitable for the activity of plant cell wall-degrading enzymes. Prusky et al. (2001) reported that some pathogenic fungi can increase their virulence by altering the ambient pH of their hosts. The ambient pH is a regulatory cue for processes associated with fungal infection, virulence, and pathogenicity (Rollins & Dickman, 2001). Organic acid molecules secreted by acidic fungi are multifunctional, including activating virulence factors and enhancing the pathogenicity of certain fungi.

Nevertheless, the underlying mechanisms by which acidification can increase fungal pathogenicity remain to be elucidated. Concentrations of different organic acids produced by acidic fungi after infecting their hosts have been examined previously, and it has been reported that acidification processes depend on the host's carbon content and expression of genes for acidifying products (Prusky & Yakoby, 2003; Vilanova et al., 2014). Therefore, systematic understanding of the relationships between acidification and pathogenicity is crucial for further exploring the mechanisms of fungal pathogens, effectively controlling postharvest diseases and reducing postharvest losses. In this paper, we briefly review several postharvest acidic fungi and their main organic acids. Moreover, we focus on transcriptional regulation and the role of organic acids in infection of plant tissues to provide a better understanding of organic acids produced by pathogenic fungi as well as the virulence mechanisms of the pathogens.

2 | ACIDIC FUNGI AND THE PREDOMINANT ORGANIC ACIDS

The change in pH in plant tissues depends on the initial ambient pH and the host's buffering capacity (Davidzon et al., 2010). When infecting plant tissues, some fungal pathogens may secrete pH-regulating compounds to lower or increase the environmental pH to enhance infection, and such pH-regulating compounds are typically organic acids or ammonia (Davidzon et al., 2010; Rollins & Dickman, 2001). Fungi that can lower or increase the pH are termed "acidic fungi" or "alkaline fungi", respectively (Alkan et al., 2013). A large number of postharvest fungi infect their host by secreting organic acids to achieve tissue acidification. These include *P. expansum*, *P. digitatum*, and *P. italicum* (Prusky & Yakoby, 2003; Vilanova et al., 2014), *Phomopsis mangiferae* (Davidzon et al., 2010), *B. cinerea* (Manteru et al., 2003), as well as *S. sclerotiorum* (Rollins & Dickman, 2001) and other postharvest pathogens.

According to Vylkova (2017), *Aspergillus niger*, a fungal strain commonly used for fermentation and food processing, is also considered an acidic fungus. As shown in Table 1, *S. sclerotiorum* and *B. cinerea* lower the pH in their host's environment by secreting large amounts of oxalic acid (Manteru et al., 2003; Rollins & Dickman, 2001). *Penicillium* spp. mainly secrete gluconic and citric acid (Davidzon et al., 2010; Vilanova et al., 2014), whereas *P. expansum* and *P. mangiferae* mainly secrete gluconic acid when infecting fruits such as apples and mangoes (Davidzon et al., 2010; Prusky et al., 2004).

According to the previous report, the contents of organic acids decrease with reducing virulence or fungal pathogenicity (Barad et al., 2012; Kunz et al., 2006; Liang et al., 2015). Knockout of genes related to organic acid synthesis or encoding transcription factor

TABLE 1 Main acidic fungi and secreted organic acids

Pathogen	Organic acid	Host	References
Penicillium digitatum	Gluconic acid, citric acid, fumaric acid	Citrus	Vilanova et al. (2014); Zhang et al. (2021)
Penicillium expansum	Gluconic acid, citric acid, fumaric acid	Apples, pears, cherries	Barad et al. (2014, 2016); Vilanova et al. (2014)
Penicillium italicum	Gluconic acid, citric acid, fumaric acid	Citrus	Penalva et al. (2008); Prusky and Yakoby (2003); Prusky et al. (2004)
Aspergillus niger	Gluconic acid, citric acid	Grapes, apples	Prusky and Lichter (2008); Vylkova (2017)
Aspergillus carbonarius	Gluconic acid, citric acid	Grapes, peaches, pears, citrus, and nectarines	Barda et al. (2020); Maor et al. (2017)
Fusarium oxysporum	Fumaric acid	Watermelons, tomatoes, and other hosts	Kwon et al. (2007); Weert et al. (2004)
Fusarium proliferatum	Fumaric acid	Onion, leek, chives, and garlic	Palmero et al. (2010)
Phomopsis mangiferae	Gluconic acid	Mangos	Davidzon et al. (2010)
Sclerotinia sclerotiorum	Oxalic acid	Wide range of hosts including oilseed rape	Cessna et al. (2000); Liang et al. (2015); McCaghey et al. (2019); Rollins and Dickman (2001)
Botrytis cinerea	Oxalic acid	Tomatoes, apples, pumpkins, and peppers	Manteru et al. (2003); Ugolini et al. (2014)

genes will reduce organic acid accumulation, and ultimately lead to weakened fungal pathogenicity. For example, a transcription factor (pacC) mutant of *Aspergillus carbonarius* is unable to efficiently acidify growth medium or infect its host as a direct result of diminished gluconic and citric acid production (Barda et al., 2020). Moreover, a study reporting the effect of organic acids (as pH modulators) on the response of citrus fruit to compatible and noncompatible pathogens indicated that *P. digitatum* pathogenicity was enhanced only when host-tissue acidification was accompanied by the suppression of H_2O_2 (Macarisin et al., 2007). This research emphasizes the important contribution of organic acids, including citric acid, oxalic acid, and gluconic acid, in fungal colonization of fruit tissue, but also that pathogenic development did not only rely on organic acid content.

3 | BIOSYNTHESIS OF ORGANIC ACIDS IN ACIDIC FUNGI

Numerous studies have reported that organic acids secreted by acidic fungi are crucial in pathogen virulence (Barad et al., 2014; Penalva et al., 2008; Rollins & Dickman, 2001). The acidic environment produced by organic acids during pathogen attack may alter the apoplastic pH, which improves the activity of other fungal pathogenic factors such as cell wall-degrading enzymes, especially polygalacturonases (PGs), during infection (Barad et al., 2014; McCaghey et al., 2019; Prusky & Yakoby, 2003). Organic acids such as oxalic, citric, and gluconic acids are important for fungal pathogenicity. The ability of acidic fungi to produce organic acids to a certain extent determines the ability of these fungi to infect (Prusky et al., 2016).

Oxalic and citric acids are synthesized in the later stages of the tricarboxylic acid (TCA) and glyoxylate cycles (Munir et al., 2001; Papagianni, 2007). Breakdown of isocitrate by isocitrate lyase is the first stage in the glyoxylate shunt, a pathway that regenerates the TCA cycle precursors during growth. Moreover, two enzymes, glyoxylate oxidase and oxaloacetase acetylhydrolase, are involved in oxalic acid metabolism in fungi: oxaloacetate acetylhydrolase catalyses the hydrolysis of oxaloacetate to produce oxalic acid and acetic acid (Chen et al., 2010; Han et al., 2007); and glyoxylate oxidase catalyses the oxidation reaction of glyoxylic acid to produce oxalic acid (Prusky et al., 2016). Some fungi only accumulate oxalic acid through oxaloacetate, so knocking out the corresponding gene cannot produce oxalic acid. The fact that oxalic acid plays a pivotal role in pathogenic virulence is supported by a large number of experiments at the gene level. Liang et al. (2015) produced a mutant with a knocked-out oxaloacetate acetylhydrolase *OAH* gene, which was unable to synthesize oxalic acid. Inoculation tests showed a significant reduction in the mutant's virulence. Kunz et al. (2006) found that the mutant A336 of *B. cinerea*, which lacks oxalic acid, can grow in normal conditions in vitro but is nonpathogenic. A similar phenomenon was observed in *S. sclerotiorum* (Tian et al., 2016). In addition, oxalic acid can act synergistically with PG to promote infection and colonization by *S. sclerotiorum*. Observation of disease onset in sugar beet leaves after inoculation with *S. sclerotiorum* showed that tissue maceration occurred before the host was covered with mycelial growth, indicating that oxalic acid had entered the host tissue before mycelial invasion, confirming that *S. sclerotiorum* secretes oxalic acid earlier in the infection process than it secretes cell wall-degrading enzymes (Tu, 1985). This is consistent with the conclusion that oxalic acid accumulation can produce an acidic environment suitable for other pathogenicity factors and enzymatic activity (Kunz et al., 2006). However, oxalic acid production and accumulation are influenced by multiple factors such as oxalic acid dehydrogenase genes, environmental pH, and the transcription factor *pac1* (considered to be a structural homolog of *PacC*).

Gluconic acid is produced through oxidation of β-D-glucose to D-gluconolactone and H_2O_2 by glucose oxidase (GOX) (Davidzon et al., 2010). Therefore, the activity and expression of GOX are essential for gluconic acid production. Hadas et al. (2008) identified two putative genes (*GOX1* and *GOX2*) in *P. expansum* and analysed transcript expression of the *GOX* family in infected tissues, which showed that *GOX2* expression was higher than that of *GOX1*, suggesting that *GOX2* may be more important for pathogenicity. However, a new *GOX3* gene was recently identified by RNA sequencing, and its transcription was induced before that of *GOX2* (Tian et al., 2016). Further studies are needed to examine the effect of *GOX3* expression on the pathogenicity of acidic fungi. Davidzon et al. (2010) analysed the acidification process in mango infected with *P. mangiferae* and found that the expression level of *pmgox1*, encoding GOX, was 8–12-fold higher at pH 7 and 8 than that at pH 4. Moreover, high transcription levels of *pmgox1*, and gluconic acid and H_2O_2 content were found in infected fruit tissues, which confirmed that GOX contributed to acidification of plant tissues by *P. mangiferae*. These observations highlight the importance of acidification for pathogenicity of *P. mangiferae*. Acidification of tissues by gluconic acid is important for the induction of biosynthesis and accumulation of patulin and the fungus' pathogenicity in *P. expansum* (Barad et al., 2014). When *P. expansum* infects plant tissues, gluconic acid, as the sole carbon source, induces accumulation of patulin. Therefore, accumulation of gluconic acid as a precursor may directly regulate patulin synthesis, thereby regulating the activation of transcription factor PacC as well as that of other pathogenicity factors. These factors also contribute to the colonization of *P. expansum* in plant tissues (Barad et al., 2014). It has been suggested that gluconic acid may be metabolized via glycolytic pathways to pyruvate, which in turn is metabolized to patulin (Sumitra et al., 2006).

The alteration of the host tissue pH through exogenous elicitors also confirms that organic acids are important for pathogenic fungi. Accumulation of exogenous oxalic acid may effectively inhibit the development of blue mould disease in kiwi fruit and reduce patulin accumulation, thereby improving fruit storage quality (Zhu et al., 2016). Ammonia activates PacC and patulin in acidified environments when apples are infected by *P. expansum* (Barad et al., 2014). Artificial acidification of host tissues can increase the virulence of certain pathogens. Artificial addition of 100 mM sodium bicarbonate ($NaHCO_3$) to fruit can increase the pH and decelerate the spread of fruit disease spots during infection by *Colletotrichum musae*

(Costa & Gunawardhana, 2012). Zhou et al. (2018) found that essential oil extracts can reduce organic acid secretion by *P. expansum* in vitro, thereby reducing its infectious ability. Accumulation of exogenous organic acids can be used as a regulator of acidic pH response processes, which in turn affects the environmental pH signal pathway and gene transcript expression, which may be a viable strategy to prevent detrimental effects of such postharvest pathogens (Keel et al., 2009). Regarding organic acids, the regulation of organic acid synthesis or identifying elicitors for postharvest control of fruit acidity will be promising approaches to prevent postharvest fruit diseases.

4 | REGULATION OF ORGANIC ACID PRODUCTION

The ambient pH regulates differential selective expression of pathogenic factors in pathogens via pH signalling pathways, which allows pathogens to infect their hosts more effectively (Alkan et al., 2013; Gomes et al., 2020). Considering different host environments, many fungi adapt and develop environment-sensing mechanisms to adjust the ambient pH to best suit their pathogenicity. Fungal response is achieved by regulating the amount of incoming and outgoing ions (Mingot et al., 2001). The production of organic acids by commercial filamentous fungi using biotechnology is increasingly important (Sauer et al., 2008), but many aspects of organic acid excretion by postharvest pathogenic fungi are still poorly understood (Pamela et al., 2012). This is partly due to the multitude of influencing factors such as host medium composition and conditions such as sugar content and pH. In many organisms, acidification is induced under carbon excess, such as 175 mM sucrose (the most abundant sugar in fruits) (Bi et al., 2016). Ambient pH strongly influences growth and organic acid excretion in postharvest fungi. From a biotechnological perspective, the maximal citric acid production by *A. niger* occurs when the pH is lower than 2, gluconic acid production at pH 4.5–6.5, and oxalic acid excretion above pH 5 (Pamela et al., 2012; Papagianni, 2007).

Ambient pH levels refer to the pH of the environment wherein microorganisms survive and proliferate. Several microorganisms grow over a wide pH range and their gene expression is tailored to the pH of their growth environment, so that permeases, secreted enzymes and metabolites are synthesized only at pH values in which they can function (Penalva et al., 2008). Ambient pH levels are important in determining the pathogenicity of the pathogen to successfully invade and colonize (Prusky et al., 2016). Under alkaline conditions, the synthesis of organic acids is triggered in postharvest pathogenic fungi. It has been reported that there are seven dedicated genes involved in pH regulation in *Aspergillus nidulans*, including *pacC*, *palA*, *palB*, *palC*, *palF*, *palH*, and *palI* (Penalva et al., 2008). PacC is a transcription factor responding to pH, and contains three Cys_2His_2 zinc finger motifs, recognizing the core region of 5′-GCCARG (Ment et al., 2015). Under acidic conditions, PacC (72-kDa $PacC^{72}$) is not active, but under alkaline conditions, the $PacC^{72}$ undergoes two successive proteolytic cleavages. In the first step, $PacC^{72}$ is hydrolysed into $PacC^{53}$. The second step of the catalysis reaction is mainly regulated by PalB, independently of pH. Under the action of a protease, $PacC^{53}$ is cleaved to $PacC^{27}$, which has the function of inhibiting or activating genes (Arst & Penalva, 2003). Therefore, changes in environmental pH have a significant effect on the expression of PacC, eventually resulting in a sophisticated system of preferred acids and alkalis as a function of ambient pH (Andersen et al., 2009).

The ambient pH signalling pathway mediated by the transcription factor PacC has been intensively studied, and is known as the pal pathway in filamentous fungi (Penalva et al., 2008). PacC has been reported to be a virulence factor of numerous fungi. However, under acidic conditions, PacC acts as a negative regulatory factor, which is important for the invasion of *Fusarium oxysporum* and *Fusarium graminearum* (Chen et al., 2018; Merhej et al., 2011). PacC, as a transcription factor, regulates adaptations to the environment, secondary metabolism processes, and virulence in many fungal pathogens. Recent research reported the regulation of organic acids by PacC in the pathogenesis of postharvest fungi such as *P. expansum* (Barad et al., 2016; Chen et al., 2018) and *P. digitatum* (Zhang et al., 2013). These studies demonstrated that PacC plays an important role in the production of organic acids by postharvest fungi via regulation of the expression of genes for enzymes involved in the biosynthesis and degradation of organic acids such as GOX and oxaloacetate hydrolase. In *S. sclerotiorum*, *pac1* acts as a pH-sensing transcription factor that accumulates in response to elevated environmental pH and activates *pac1*-mediated downstream signalling, which in turn facilitates oxalic acid biosynthesis (Rollins & Dickman, 2001). This study demonstrated the production of oxalic acid mediated by pH using a Δ*pac1* mutant, suggesting that the production of pathogenic factors is a consequence of ambient pH regulation and that its accumulation may help regulate the acidification process. Barad et al. (2014) constructed two *PacC* gene mutants by down-regulating *PacC* expression using RNA interference (RNAi) technology. They found production of gluconic acid in the mutants decreased by 63% and 27%, and accumulation of patulin also reduced. Chen et al. (2018) found that virulence of the Δ*PePacC* mutant was obviously reduced when infecting pear and apple fruits. PePacC can activate different target proteins such as calreticulin, glucose oxidase, and sulphate adenylate transferase, which confirms that the PacC transcription factor is associated with *P. expansum* virulence. Similarly, deletion of PacC homologs in pathogenic fungi such as *P. digitatum* (Zhang et al., 2013), *Colletotrichum acutatum* (You et al., 2007), and *Colletotrichum gloeosporioides* (Miyara et al., 2008) can reduce the virulence of plant pathogens. However, there are also some reports claiming that PacC has no significant effect on the pathogenesis of certain fungi. The endoPG family, including PacC homologs, in *B. cinerea* exhibit differential expressions at various pH conditions, but there is no evidence suggesting that PacC can act as a transcription factor affecting the pathogenicity of *B. cinerea* (Christine et al., 2018; Gomes et al., 2020). Additionally, PacC negatively affected the pathogenicity of *F. oxysporum* and *F. graminearum*, suggesting that PacC is a crucial element of a complex regulatory network that controls the virulence of different fungal pathogens (Caracuel et al., 2003).

Cyclic adenosine mono-phosphate (cAMP) and the mitogen-activated protein kinase (MAPK) signal transduction pathway can regulate the formation of fungal appressoria (Franck et al., 2013; Zhao et al., 2005). Recognition of the external pH environment and further infection of the host by fungi are inseparable from intracellular signal regulatory systems, such as the cAMP and MAPK pathways. Increased levels of endogenous or exogenous cAMP can lead to oxalic acid accumulation in *S. sclerotiorum* (Rollins & Dickman, 2001). cAMP-mediated signalling interacts with environmental pH signalling, thereby regulating oxalic acid production and sclerotium development (Alkan et al., 2013). MAPK signalling cascades act in synergy with pathways such as cAMP/PKA or environmental pH signalling in *Candida albicans* in response to external pH (Leberer et al., 2001).

5 | MECHANISMS OF ACTION OF ORGANIC ACIDS INVOLVED IN FUNGAL PATHOGENICITY

5.1 | Oxalic acid

Oxalic acid is one of the key factors in the pathogenesis of *S. sclerotiorum* and *B. cinerea* (Cessna et al., 2000; Prusky & Lichter, 2008). Oxalic acid plays a similar role in the pathogenesis of *B. cinerea* as in that of *S. sclerotiorum* (Cessna et al., 2000; Manteru et al., 2003). However, the interactions between *S. sclerotiorum* and its hosts have been studied in more detail. The effect of oxalic acid on *S. sclerotiorum* pathogenesis can be summarized as follows: (a) Oxalic acid promotes the accumulation of initial acid and reduction of extracellular pH at the site of infection. The high concentration of oxalic acid reduces the extracellular pH to 4–5 at the site of infection, which enhances the activity of cell wall-degrading enzymes such as PG, cellulase, hemicellulose, and pectinase (Cessna et al., 2000; Rollins & Dickman, 2001). In addition, the acidified environment inhibits the activity of enzymes associated with plant resistance to disease such as phenylalanine ammonia-lyase and polyphenol oxidase. The decrease in pH also influences the transcription of other pathogenicity factor genes, such as *pg1* (Prusky & Lichter, 2008). (b) Chelation of metal ions from plant tissues by oxalic acid is toxic to host tissues. Oxalic acid chelates Ca^{2+} to produce insoluble calcium oxalate crystals, which disrupt cell walls and block plant ducts, causing wilting in plant hosts (Cessna et al., 2000; Prusky & Lichter, 2008). Calcium oxalate also causes destabilization of host pectin polymers, thus reducing access and sensitivity to pectinolytic enzymes produced by *S. sclerotiorum* (Rollins & Dickman, 2001). Chelation of Mg^{2+} by oxalic acid leads to failure of normal chlorophyll synthesis and disruption of ribosomal functions (Kabbage et al., 2013). (c) Oxalic acid alters redox reactions in plant tissues and inhibits defence responses. Moreover, oxalic acid inhibits the oxidative burst, callose deposition, and other early defence reactions in the host by generating a reducing environment, thus reducing the oxidative damage to *S. sclerotiorum* by plant tissues during invasion. Oxalic acid can impede signal transduction in the oxidative burst pathway of the host and inhibit the synthesis or activation of oxidase accumulation during the oxidative burst (Cessna et al., 2000; Williams et al., 2011). The acidic environment produced by oxalic acid accumulation hampers the production of H_2O_2 and inhibits the oxidative burst in the host (Kim et al., 2008). (d) Oxalic acid can induce the production of reactive oxygen species (ROS) in plant tissues and can cause programmed cell death (PCD) in plants (Williams et al., 2011). When *S. sclerotiorum* invades the host, oxalic acid can stimulate early anion and K^+ loss from plant cells, thereby inducing cell dehydration and contraction, and causing a PCD response. When the host's early defence response is down-regulated, a saprophytic nutrient environment may develop within the host, which elicits the production of ROS-inducing factors that promote PCD and disease development in infected tissues (Li et al., 2016; Veluchamy et al., 2011; Williams et al., 2011). (e) Oxalic acid promotes successful infection by *S. sclerotiorum* through inhibiting plant cell autophagy and inducing apoptosis over large areas. The mechanisms of action of organic acids involved in fungal pathogenicity are described in Figure 1.

5.2 | Gluconic acid

Penicillium spp. and *P. mangiferae* can enhance their virulence by secreting gluconic acid, which affects the host's environmental pH. Gluconic acid enhances fungal virulence in the following ways: (a) Gluconic acid reduces the host pH. *P. mangiferae* decreases the pH in mango and grapefruit tissue from 5.1 and 4.1 to 3.8 and 2.5, respectively. Gluconic acid is the predominant organic acid at the infection site. Inoculation with *P. digitatum* and *P. expansum* can decrease the pH in the pericarp of oranges and apples by 2 and 0.5 units, respectively (Vilanova et al., 2014). A similar phenomenon has been observed in navel oranges and grapefruits by Prusky et al. (2004), who also found that gluconic acid and fumaric acid might be responsible for the low pH of decaying apple tissue. (b) Gluconic acid produces the acidic environment required for cell wall-degrading enzyme activity. Local acidification of plant tissue increases the virulence of *P. expansum* and facilitates functioning of cell wall degradation balance, which may affect the stability of cell membranes and cell wall pectin polymers (Prusky et al., 2016). (c) Gluconic acid contributes to toxin accumulation. Gluconic acid may be converted into pyruvic acid through the sugar degradation pathway and is further metabolized into patulin (Hadas et al., 2008).

5.3 | Citric acid

Citric acid secreted by *Penicillium* spp., *A. niger*, and *Geotrichum candidum* during infection of fruits improves the virulence of the fungal pathogens. Citric acid increases fungal pathogenicity in the following ways: (a) Citric acid lowers the pH of the host tissue. Citric acid secreted by *P. digitatum* and *P. expansum* can reduce the pH in the pericarp of oranges and apples by 2 and 0.5 units, respectively (Vilanova et al., 2014). (b) Citric acid produces the acidic environment required

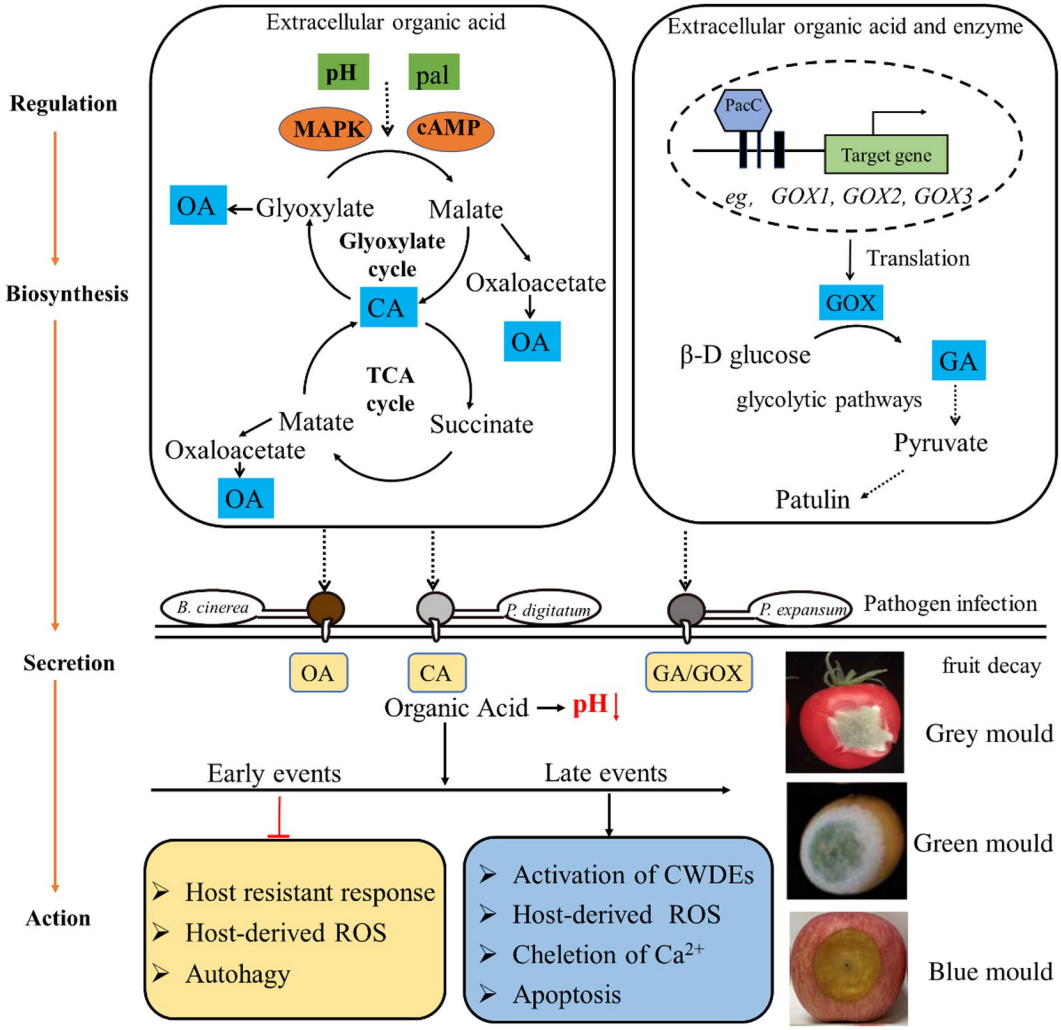

FIGURE 1 Action mechanism of the main organic acids secreted by postharvest pathogenic fungi (*Botrytis cinerea*, *Penicillium digitatum*, *Penicillium expansum*) during infection of host tissues. CA, citric acid; CWDE, cell wall-degrading enzyme; GA, gluconic acid; GOX, glucose oxidase; OA, oxalic acid; ROS, reactive oxygen species; TCA, tricarboxylic acid

for cell wall-degrading enzyme activity. Local acidification of plant tissues increases the pathogenicity of fungi such as *P. expansum* and facilitates the functioning of cell wall-degrading enzymes at optimal pH conditions, thereby creating an optimal environment for the activity of PG produced by the fungus (Prusky et al., 2001; Prusky & Yakoby, 2003). (c) Citric acid inhibits the production of H_2O_2 in host tissues (Macarisin et al., 2007). (d) Chelation of Ca^{2+} by citric acid weakens the cell wall functions of the host and leads to cell death. Accumulation of citric acid reduces Ca^{2+} activity between plant cells, altering the mineral balance, and affecting the stability of cell membranes and cell wall pectin polymers (Liu et al., 2020). The functions of citric acid involved in fungal pathogenicity are also illustrated in Figure 1.

5.4 | Other organic acids

F. oxysporum can decrease extracellular pH and secrete fumaric acid in watermelon, tomato, and other hosts, which can be directly involved in pathogenesis and promote the expression of pathogenicity-related acidification genes (Caracuel et al., 2003). Vilanova et al. (2014) reported that *P. digitatum* and *P. expansum* secrete ascorbic acid, fumaric acid, and succinic acid in addition to citric acid and gluconic acid in the process of infecting hosts. However, it is noteworthy that according to the organic acid content in fruit tissue at the site of infection, not all secreted organic acids necessarily contribute to fungal virulence and pathogenicity.

6 | CONCLUSIONS

Numerous studies have reported that acidic fungi can increase their pathogenicity by secreting organic acids and lowering environmental pH. Pathogenic mechanisms involving acidification are considerably complex, and they cannot be separated from fungal adaptation, fungal–host interactions, and the host's ability to resist invasion. Moreover, acidification processes are influenced and regulated by

several factors and signals, requiring further research. For example, (a) numerous species of postharvest pathogenic fungi occur in fruits and vegetables, but only a few pathogenic fungi exerting acidification effects have been identified, and novel acidic fungi must be identified and studied to improve respective databases; (b) the precise mechanisms mediating the secretion of organic acids from fungal cells remain to be elucidated, and the links between pH sensing and known pathogenic signalling modules must be investigated further; (c) comprehensive information on pathogenic mechanisms and regulatory networks of organic acid molecules during acidification need to be demonstrated; and (d) the transport and metabolic pathways of important organic acids during pathogenesis have not been comprehensively examined. In-depth understanding of fungal–host interactions with regard to organic acids may be of interest for future research, including aspects such as fungal species that exert acidification, types of secreted organic acids, and pH signalling pathways. These results would help in understanding the mechanisms of pH changes on pathogenicity, and may provide theoretical and technical guidance for developing new disease control strategies and reducing postharvest losses by disrupting acidification processes.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed.

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