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# Advances and Strategies for Controlling the Quality and Safety of Postharvest Fruit

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ABSTRACT

Fresh fruits are highly valued by consumers worldwide, owing to their delicious flavors, abundant nutrients, and health-promoting characteristics, and as such, fruits make up an important component of a healthy diet. The postharvest quality and safety of fresh fruit involve complex interactions among the fruit, environmental factors, and postharvest pathogens. Efficient regulation of fruit senescence and pathogen resistance, as well as disease-causing abilities of postharvest pathogens, is critical to understanding the fundamental mechanisms that underlie fruit quality and safety. This paper provides a comprehensive review of recent advances and currently available strategies for maintaining fruit quality and controlling major postharvest pathogens, mainly *Botrytis cinerea* and *Penicillium expansum*, which may promote sustainable and environmental-friendly development of the fruit industry.



## 1. Introduction

Fresh fruits are abundant in organic acids, sugars, vitamins, minerals, and other health-promoting constituents and, thus, represent an indispensable component of daily human diets [1,2]. With continuous improvements in living standards and significant transitions in the consumption concepts of consumers, the contribution of the fruit industry to the world economy has increased dramatically over the past several decades. According to the latest statistics from the Ministry of Agriculture of China and Rural Affairs of the People's Republic and the Food and Agriculture Organization of the United Nations, China has continuously ranked first in the world for the annual production of fresh fruit for more than two decades [3]. However, serious quality deterioration and postharvest losses inevitably occur, owing to a variety of reasons during both pre-harvest and postharvest stages, especially in developing countries, and about a third of the fresh fruit produced in developing countries fails to reach the tables of consumers. Among the various factors that affect such losses, intrinsic physiological senescence and infection by fungal pathogens are the most important [4]. Even though the application of synthetic chemicals remains the most convenient and economical method for controlling postharvest loss, the persistent application of chemical pesticides has resulted in public concerns over both environmental contamination and food safety. However, the toxic secondary metabolites produced by fungal pathogens also threaten consumer health. Therefore, it is necessary to update the traditional techniques used for pathogen management and to develop precise methods for efficiently regulating fruit senescence and resistance, as well as the disease-causing abilities of pathogens. Based on currently available advances in the studies on controlling the quality and safety of postharvest fruit, this review mainly focuses on the strategies to employ molecular basis underlying quality maintenance and antioxidant pathways in fruit, further explore safe substances synergistically regulating fruit senescence and antioxidant capacity during the postharvest stage, and dissect potential targets modulating pathogenicity and toxin production in fungal pathogens (Fig. 1).

## 2. Fruit ripening, senescence, and resistance responses

### 2.1. Development and maintenance of fruit quality

Fruit ripening and senescence are highly complex and ordered physiological processes that are directly related to the formation

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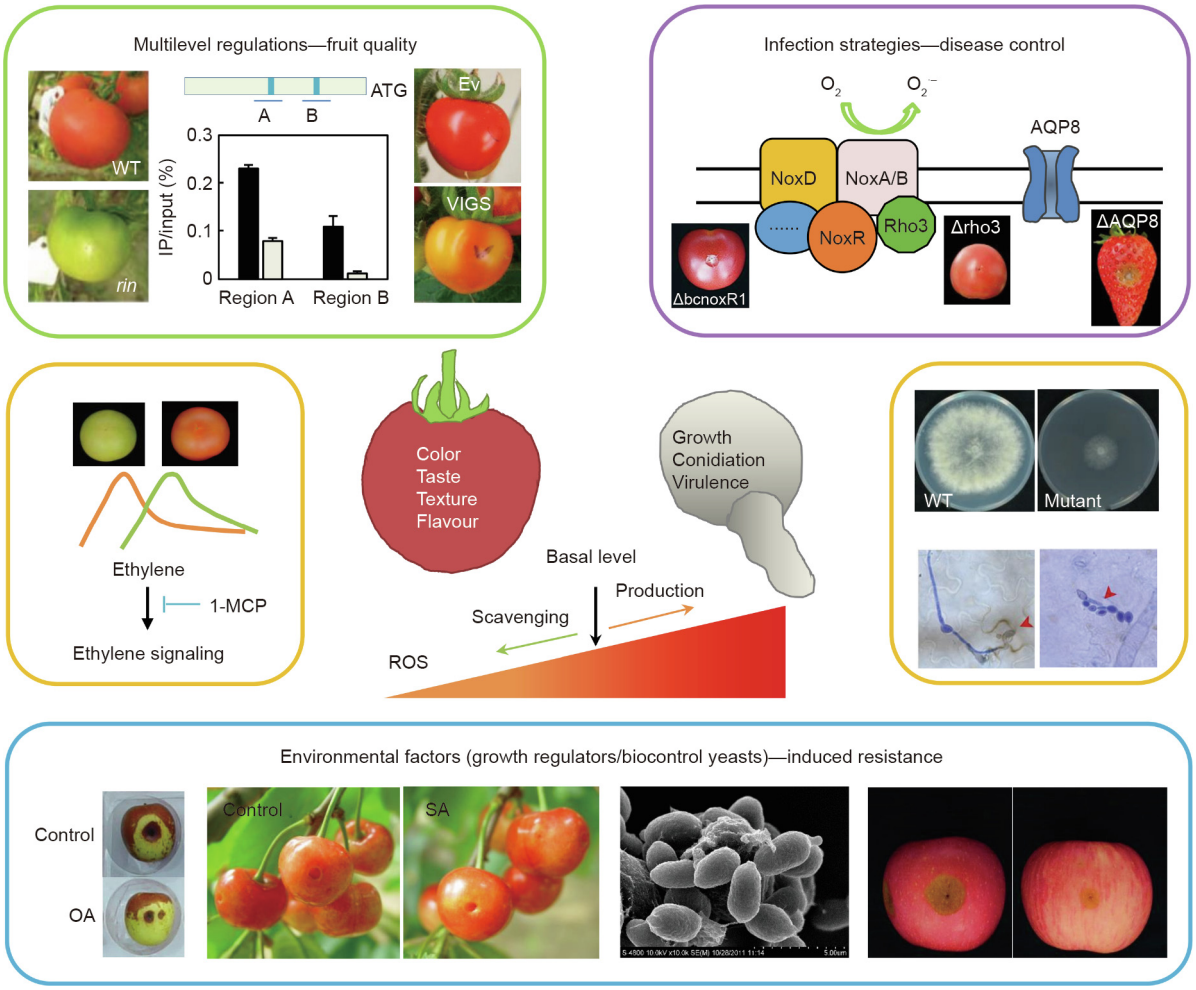


**Fig. 1.** Fundamental aspects involving fruit, fungal pathogens, and exogenous factors during postharvest stage. 1-MCP: 1-methylcyclopropene; SA: salicylic acid; OA: oxalic acid; NHS: non-heat shock; ROS: reactive oxygen species; WT: wildtype; *rin*: ripening inhibitor; Ev: empty vector; VIGS: virus-induced gene silencing; IP: immunoprecipitation; AQP: aquaporin.

and maintenance of fruit quality [2,5]. These processes involve the spatiotemporal reprogramming of multiple genes that ultimately trigger subtle variations in color, flavor, aroma, texture, and other quality attributes [6]. Ripe fleshy fruits generally exhibit greater susceptibilities than premature fruits to postharvest disease and decomposition [7], and greater pathogen susceptibility can lead to high losses in fruit yield, which is especially significant when losses occur when fruits possess their highest commodity values, whereas it also facilitates seed dispersal from ripe fruit at the same time [8]. Therefore, developing a more comprehensive understanding of ripening-related events that are associated with increases in pathogen susceptibility could significantly affect future fruit yield and subsequent commercialization by improving the production of high-quality fruit at appropriate maturity stages and by improving fruit pathogen resistance and shelf life.

Many climacteric fruits (e.g., tomatoes, apples, and bananas) exhibit almost concurrent respiratory bursts and ethylene peaks upon the initiation of ripening [5]. Such fruits are normally harvested at relatively low maturity and are later processed to promote physiological maturation. During these processes, the use of excessive levels of ethylene can lead to rapid softening and quality deterioration. Therefore, the elaborate modulation of ethylene biosynthesis or signaling has great importance for prolonging shelf-life and for maintaining quality traits during the postharvest stage. As a competitive inhibitor of ethylene receptors, 1-methylcyclopropene (1-MCP) suppresses ethylene-related responses [9] and, thus, has been used extensively for delaying the ripening of climactic fleshy fruits [10,11]. In addition to its effectiveness in fruits with inheritent physiological differences [10,12] and resistance to fungal pathogens [11,13], 1-MCP can be used to both maintain fruit flavors, by balancing organic acid dynamics, and to regulate levels of sugars and flavor volatiles, thereby determining the organoleptic and intrinsic qualities of fruit [14]. Tomato is an economically and scientifically important crop plant, as a well-known vegetable and model plant, and is especially valuable for investigating fruit ripening and senescence. Among several well-characterized genes related to fruit ripening [15], which include *rin* (ripening-inhibitor) [16], *nor* (non-ripening) [17], *Cnr* (colorless non-ripening) [18], *Gr* (green-ripe) [19], and *Nr* (never ripe) [20], the MADS-box transcription factor RIPENING-INHIBITOR (RIN) has been extensively investigated in regard to its role in activating the transcription of ripening-related genes. In one such study [21], chromatin immunoprecipitation (ChIP) was used to identify 241 potential RIN target genes that could be involved in the determination of fruit quality. For example, genes related to specific aroma production (e.g., *TomloxC* and *ADH2*) [22], ubiquitin-proteasome pathways (e.g., *SlUBC32* and *PSMD2*) [23], and cell wall modeling and carbohydrate modifications [24]

have all been identified as RIN-binding targets. However, several recent studies have demonstrated that *rin* is a gain-of-function mutation, rather than a null mutation, that produces a protein that actively represses ripening [25,26]. Thus, the functions of RIN are still far from clear, and mutations of multiple alleles should be examined to elucidate the functions of specific genes.

The ripening of fleshy fruits involves simultaneous changes in fruit biochemistry and physiology [27], especially increases in sugar contents and decreases in organic acid levels [27,28]. However, the metabolic changes of non-climacteric fruits during ripening are much less exaggerated than those observed in climacteric fruits. Transcriptomic analysis has revealed that carbohydrate metabolism is down-regulated during the middle and later stages of citrus fruit ripening and suggests that such changes are due to the up-regulation of sucrose phosphate synthase (SPS) and citrate synthase [29,30]. Meanwhile, sugar accumulation is directly regulated by the activities of sucrose synthase and SPS [31], whereas ratios of individual sugars vary significantly among species and maturation stage [32]. Previous studies have demonstrated that sucrose can signal the acceleration of ripening in non-climacteric fruits, such as strawberry [33], grape [34], and citrus [35]. Interestingly, sugar, acid, and cell turgor may function as early signals that promote abscisic acid (ABA) accumulation, whereas the FaABAR/CHLH and FaPYR1 signaling pathways may regulate sugar and anthocyanin biosynthesis *via* transcription factors, such as ABRE-binding factor and sigma factor [36]. Sucrose regulates the initiation of ABA accumulation in strawberry fruit *via* 9-*cis*-epoxycarotenoid dioxygenase 1 [37]. However, levels of organic acids are usually inversely correlated with those of sugars. When sugars accumulate as a result of starch degradation, the organic acids in young fruits decrease dramatically, which are under coordinated genetic and environmental control [38]. Malate and citrate are the most abundant organic acids in both climacteric and non-climacteric fruits. However, some climacteric fruits use malate as a substrate for respiration, whereas non-climacteric fruits continue to accumulate malate throughout the ripening process [39].

Fruit ripening also involves the adjustment of secondary metabolite levels. Flavonoids (e.g., phenolics and anthocyanins), which are mainly biosynthesized via phenylpropanoid pathway, are major secondary metabolites that directly affect the quality and economic value of fruits. Flavonoid biosynthesis is closely linked to key enzymes that are transcriptionally controlled by MYB transcription factors, MYC-like basic helix-loop-helix (bHLH) transcription factors, and WD40-repeat proteins [40,41]. The expression patterns and the DNA-binding specificity of MYB and bHLH transcription factors determine the gene subsets that are activated, whereas WD40 protein functions as a common transcription factor in the regulatory MYB-bHLH-WD40 (MBW) complex [40]. Some fruits accumulate anthocyanins in both their flesh and skin, whereas others only accumulate anthocyanins in their skin, and anthocyanin synthesis is predominantly controlled by developmental cues. In contrast, other fruits accumulate anthocyanins in their skin in response to environmental stimuli, such as light, temperature, drought, and mechanical injury, and thus the composition and quantities of anthocyanins may vary significantly under different environmental conditions [42,43]. The mechanisms underlying the environment-specific accumulation of anthocyanins in fruits have attracted serious interest [39,41]. However, studies have yet to examine key players in flavonoid production or to decipher the functions of flavonoids in fruit-pathogen interactions.

### 2.2. Oxidative stress and induced anti-oxidative capacity

Even though reactive oxygen species (ROS) play important roles in a variety of crucial signaling pathways, in response to developmental cues and environmental stimuli, they are also produced as harmful by-products of oxygen consumption [2,44]. Indeed, ROS are fundamentally beneficial molecules that modulate a variety of cellular processes, but in fruit, the oxidative stress caused by excessive ROS accumulation can trigger physiological deterioration pathways [45–47]. Because mitochondria are a major source of intracellular ROS generation, specific mitochondrial proteins in fresh fruit, such as voltage-dependent anion-selective channel (VDAC) proteins, aconitase, and certain antioxidant proteins (e.g., manganese superoxide dismutase), are prone to oxidative damages, especially under the unfavorable conditions that occur during fruit storage and transport, and such damage can disturb mitochondrial functions and eventually cause the deterioration of fruit quality [48]. Under oxidative stress conditions, protein carbonylation occurs concurrently with the interference of VDAC function and impairments in the catalytic activities of antioxidant enzymes, which further promotes the generation of superoxide anion radicals in mitochondria [2,48]. Importantly, the reduction of ROS levels by lowering environmental temperature has been reported to alleviate mitochondrial carbonylation levels and, thus, the process of fruit senescence [49]. The reduction of ROS contents by storage under low oxygen levels (2%–5%) can effectively postpone fruit senescence, whereas exposure to high $H_2O_2$ levels have been reported to have the opposite effect [50]. Collectively, cellular ROS homeostasis mediates the responses of fruits to adverse environmental factors, mainly by mediating antioxidant capacity, mobilizing phytohormones (for example, salicylic acid (SA), jasmonic acid, and nitric oxide), priming phospholipid signaling and other defense responses, and enhancing the remodeling of cell walls and other physical barriers [51].

The induction of intrinsic anti-oxidative capacity and disease resistance in fruit is also an efficient strategy for maintaining fruit quality and for alleviating postharvest deterioration. The mechanisms that underlie such strategies include ① inducing the production of proteins or metabolites related to disease resistance in fruit, such as pathogenesis-related proteins and certain phenol metabolism enzymes, including phytoalexins, chitinases, phenylalanine ammonia lyase, β-1,3-glucanase, and phenolics; ② alleviating oxidative injuries on proteins; and ③ reinforcing cell wall barriers. As a crucial signaling molecule for the activation of defense responses, SA activates systemic acquired resistance and further protects plants, including harvested fruit at postharvest stage, from both biotic and abiotic stresses [52]. Levels of antioxidant enzymes are significantly induced in peach and sweet cherry fruit upon exogenous SA application, which suggests that antioxidant proteins (e.g., catalase and glutathione transferase) make up at least a part of fruit defense responses [52,53]. Similarly, oxalic acid has been reported to suppress ethanol production and ethylene metabolism in jujube fruits, while simultaneously inducing the expression of defense response proteins, ultimately postponing fruit senescence and improving disease resistance [54].

### 2.3. Current gaps in studying postharvest fruit quality maintenance

Many studies of tomato fruit ripening have demonstrated that mutations can be more complex than initially expected, such as in the case of dominant-negative or gain-of-function mutations. Hence, it is tractable and reasonable to use multiple alleles when investigating ripening-related phenotypes with clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated endonuclease 9 (CRISPR/Cas9) technology. Moreover, because ripening genes may function through either up- or down-regulation, as reported by Lü et al. [6], different fruits can be expected to improve rapidly through the addition or deletion of relevant transcription factor-binding motifs from promoter regions. Therefore, a holistic analysis of epigenetic modification

sites that are relevant to gene expression profiles and fruit quality traits could reveal novel candidates for future breeding studies. In summary, a more comprehensive experimental design that involves multiple regulatory levels (e.g., transcriptional networks, posttranscriptional regulation, and epigenetics) which requires data mining and high-throughput approaches, may improve the current understanding of fruit quality maintenance.

## 3. Mechanisms and control of postharvest pathogenesis

Infection by fungal pathogens is another major threat to the global food chain supply, and great efforts have been made to explore economical, effective, and safe measures for controlling postharvest diseases [4]. The application of synthetic fungicides remains the most efficient method, owing to its convenience and low cost. However, the frequent and long-term application of fungicides has resulted in extensive public concerns over food safety and environmental risks. Moreover, the appearance of fungicide-resistant strains and the fungicide residues in environment are always resulted from unreasonable fungicide application, whereas this situation further prompt researchers to make efforts in exploring safe alternatives from natural sources.

### 3.1. Identifying potential targets of pathogenicity

The majority of postharvest pathogens are necrotrophic fungi, which were originally viewed as unsophisticated pathogens that lack effectors and kill host cells using cell wall-degrading enzymes (CWDEs) or mycotoxins [7]. However, new experimental evidence has demonstrated that this is not necessarily the case. For example, Zhang et al. [55] reported that a receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1 in *Arabidopsis* could recognize an endopolygalacturonase secreted by *Botrytis cinerea* (*B. cinerea*), which suggested that receptors and receptor-like proteins on the plasma membranes of host cells recognize secretory proteins as microbe-associated molecular patterns and, thereby, modulate the innate immunity of hosts. In addition, recent studies of small RNAs (sRNAs) [56] have revealed the occurrence of active communication between plant cells and *B. cinerea* and have endowed sRNAs with novel functions as effectors, suggesting that sRNAs may also function as virulence factors during the interaction. Furthermore, Weiberg et al. [56] reported that certain *B. cinerea* sRNAs could migrate into plant cells and use RNA interference system in hoststo silence specific genes involved in host cell immunity. However, plants are also able to employ extracellular vesicles to introduce sRNAs into *B. cinerea* and subsequently suppress the expression of genes related to pathogenicity [57]. These new findings further broaden the current understanding of the pathogenicity of postharvest pathogens, which indicates the importance of directing future research efforts toward understanding the interactions of fruit pathogens and host cells. Although some state-of-art techniques (e.g., surface plasmon resonance–tandem mass spectrometry, split-ubiquitin yeast two-hybrid assay, and single molecular tracking of membrane proteins) can be used to dissect the infection machinery of fungal pathogens and have been reportedly used to identify membrane protein interactions, the mechanisms underlying pathogenicity of major postharvest pathogens are still far from clear.

Many studies have demonstrated that ROS-generating systems and related components are involved in the vegetative growth and virulence of *B. cinerea* [58,59]. Among the multiple components of such systems, BcNoxA and BcNoxB are homologs of human catalytic subunit gp91$^{phox}$ and possess both transmembrane and catalytic domains [60,61]. More specifically, BcNoxD interacts directly with BcNoxA as a homolog of mammalian adaptor protein p22$^{phox}$, whereas another transmembrane protein, BcPls1, functions as a p22$^{phox}$ homolog in the BcNoxB complex [62]. Importantly, BcNoxR functions as a core regulator by regulating the catalytic functions of BcNoxA and BcNoxB [61,62]. However, phenotypic analyses have shown that deletion mutants of known different Nox subunits exhibit defects in sclerotia formation, hyphae growth and morphology, and appressorium-mediated penetration [61,63–65], thereby reducing pathogenicity. These results suggest that Nox subunits, in addition to their role in the maintenance of cellular ROS production and distribution, also contribute to the differentiation and developmental processes of *B. cinerea* [61,62]. Thus, it is both feasible and promising to modulate cellular ROS homeostasis in postharvest pathogens, ultimately suppressing fungal growth and pathogenicity [2].

Furthermore, the host specificity of *Penicillium expansum* (*P. expansum*) and *Penicillium digitatum* (*P. digitatum*) has been reported to involve genomic variation [66]. For example, the relatively lower genetic variability of *P. digitatum*, when compared to that of *P. expansum*, may explain the specificity of *P. digitatum* for citrus fruits [66,67]. However, specific environmental context can also affect *P. expansum* virulence. For example, a pH-responsive transcription factor, PePacC, was reported to regulate virulence and the synthesis of patulin (PAT), which plays an important role in responses to environmental pH [68]. In addition, screening of a transfer DNA (T-DNA) insertion library revealed that a secretory protein, Blistering1, also modulated the virulence of *P. expansum* [69].

### 3.2. Biological control of postharvest pathogens

Antagonistic microorganisms are safe and effective for the biological control of postharvest diseases in fruit [70], and research over the past two decades has made significant progress in screening antagonistic microorganisms, elucidating antimicrobial mechanisms, and improving biocontrol efficacy. As a result, many yeast strains have been documented as effective biocontrol agents against postharvest diseases [71–73], and such strains are generally considered superior to other agents as a result of their high efficacy toward multiple pathogens, Wide applicability in practice, low nutrient requirements, excellent compatibility with other measures, and high survival rates under unfavorable conditions [1]. The modes of action for these organisms include competition for habitat and nutrition, biofilm formation, and the metabolism of antifungal substance and volatiles [74]. Among the currently available isolates, several representative species (e.g., *Rhodotorula glutinis*, *Cryptococcus laurentii*, and *Pichia caribbica*), have demonstrated effectiveness against postharvest pathogens [75–80]. However, future research efforts should focus on improving the biocontrol efficacy of currently available strains, and the potential for biocontrol organisms to act synergistically with other effective measures should be explored.

### 3.3. Screening for biologically active substances from safe natural sources

Generally regarded as safe substances and natural substances (e.g., flavonoids, phenolics, terpenes, and their derivatives) from natural sources (e.g., plants or microorganisms) have also attractive extensive research interest. Owing to their natural steric structures, such substances have the potential to be developed into safe fresh-keeping agents. Indeed, a variety of exogenous substances (e.g., cinnamic acid [81], trisodium phosphate [82], methyl thujate [83], chitosan [84], and natamycin [85]) have been reported to be capable of improving resistance to fungal pathogens. The efficacies

of these substances in harvested fruit can partly be attributed to increases in antioxidant capacity, induced phospholipid signaling, and cell wall-related defenses. However, impairments to membrane integrity, ROS-induced oxidative stress, and the induction of autophagic activities in fungal cells also play important roles [86]. Further studies are needed to elucidate the mechanisms that underlie the activities of these substances and to determine whether substances with different targets can be combined to achieve synergistic action.

### 3.4. Challenges of studying postharvest fruit virulence factors

The current literature suggests that the pathogenesis strategies used by most fungal pathogens rely on their capacity to modulate environmental pH conditions, thereby achieving host cell wall degradation and relieving oxidative bursts [56,68,69]. Therefore, currently feasible measures combating the fungal pathogenesis of postharvest fruit include the investigation of spatiotemporal changes in the secretome profiles of pathogens when they interact with their hosts, T-DNA insertion library screening, phenotypic analysis of deletion mutants, and protein interaction analysis, among other endeavors. The analysis of either *in planta* or *in vivo* samples during early infection may also provide novel information about potential virulence factors. In addition to secretory proteins, small RNAs and mycotoxins are also emerging as potential effectors or virulence factors, and understanding of the specific roles for these virulence factors may provide new possibilities for unraveling the mechanisms that underlie fruit-pathogen interactions. Under natural conditions, the capacity of pathogens to infect fruits is largely dependent on specific environmental conditions, including ambient pH, light, temperature, and nutrient availability. Therefore, such factors could be manipulated to both promote fruit resistance and suppress pathogenesis.

## 4. Toxin production and efforts for toxin reduction

### 4.1. Toxin biosynthesis routes as targets for suppression

Filamentous fungi are well known for their production of secondary metabolites [87], which are valuable to the pharmaceutical industry but detrimental to the food industry and to agricultural production [88,89]. In addition to postharvest fruit decay, fungal pathogens also produce natural mycotoxins that are frequent contaminants in the food chain that threaten human health. PAT, which is a mycotoxin produced primarily by filamentous fungi, is a common toxic contaminant of fresh vegetables and fruits, including apples, pears, and peaches [90–92].

Given the high cost of eliminating or reducing PAT contamination in the food chain, it would be both practical and economically beneficial to elucidate PAT biosynthesis pathways, which could then be used to directly prevent PAT contamination by blocking the contaminant at its source. Gene clusters involved in PAT synthesis have been reported in several filamentous fungi, such as *Aspergillus clavatus* [93], *Penicillium griseofulvum* [94], and *P. expansum* [95–97], and the analysis of corresponding *P. expansum* knock-out mutants suggests that all 15 genes (*PePatA*–*PePatO*) in a PAT-related gene cluster were necessary for PAT biosynthesis, whereas the deletion of various genes in the aflatoxin gene cluster had no significant effect [98,99]. Notably, the production of PAT by *P. expansum* was completely inhibited by knocking out eight genes that encode catalyzing enzymes [99], thereby indicating that the genes were indispensable for normal PAT production. After the determination that PePatL, a putative C6 transcription factor, was specific to the PAT biosynthesis pathway [97], further analyses successfully elucidated the PAT biosynthesis pathway in full. In addition to pathway-specific transcription factors, some global transcriptional factors (e.g., VeA, VelB, and VelC) are also involved in the regulation of mycotoxin production, and such proteins represent potential targets for mycotoxin suppression [99].

### 4.2. Efforts for reducing toxin production

Progress in the reduction or elimination of PAT from the food chain is relatively slow, and either persistent cleaning or the removal of decayed tissues remain the most efficient and economical measures of PAT reduction [100]. Fermentation with yeast strains can also reduce PAT content dramatically, usually by > 90%, likely owing to the metabolism or degradation of PAT by yeast cells [101,102]. However, fermentation could affect the original flavor of fruit products, and the situation is made even more complex by sophisticated storage conditions, pre- and postharvest treatments, and physical injuries to fruit surfaces. Some natural substances (e.g., plant-derived phenolic acids) can also reduce mycotoxin production markedly [103]. For example, *p*-coumaric acid has been reported to significantly reduce the accumulation of the type-A trichothecene mycotoxins T-2 and HT-2 by about 90% and to reduce the accumulation of zearalenone by 48%–77% [103]. Thus, such substances can clearly be used as both pre- and postharvest measures for controlling the accumulation of toxins by *Fusarium* spp. and other fungal pathogens. Extensive screening for new sources (e.g., wood and bamboo waste or traditional Chinese medicinal plants) of natural flavonoids are still required, and the utilization of such resources might also be useful for reducing resource waste and environmental pollution.

## 5. Perspectives

Recent studies have made significant advances in the control of postharvest fruit quality and food safety (Fig. 2). However, natural environments are quite different from experimental postharvest conditions, particularly during transport and storage. Moreover, most laboratory experiments examine antimicrobial efficacies by producing wounds on fresh fruit, whereas the efficacies should be manifested both on the surface and interior of fruits during commercial applications. Therefore, future studies should emphasize ① additional screening for substances and microorganisms with broad anti-microbial spectra and high efficacies, ② the comprehensive study of mechanisms and modes of interactions between fruit, pathogens, and environmental factors, and ③ the combination of strategies or substances with differing modes of action in order to achieve synergistic effects.

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## Compliance with ethics guidelines

Tong Chen, Dongchao Ji, Zhanquan Zhang, Boqiang Li, Guozheng Qin, and Shiping Tian declare that they have no conflict of interest or financial conflicts to disclose.

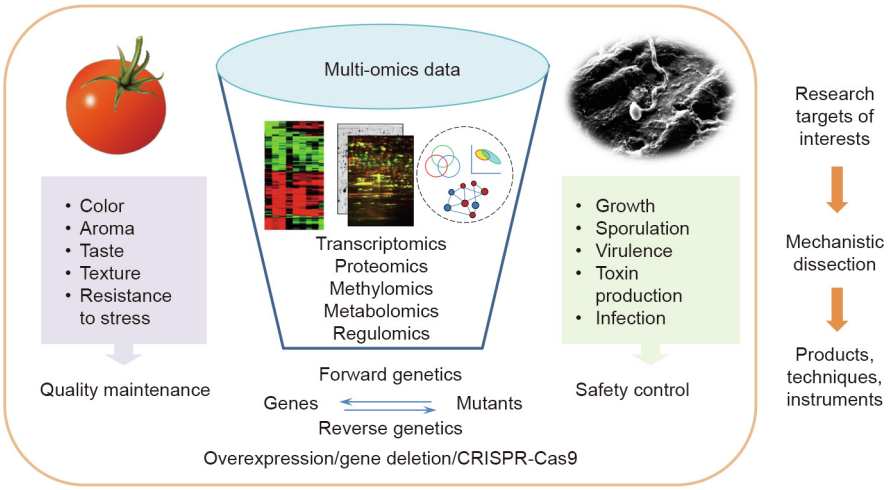


**Fig. 2.** Strategies for dissecting the mechanisms that underlie the quality maintenance and food safety of postharvest fruit.

## References


[1] Droby S, Wisniewski M, Macarisin D, Wilson C. Twenty years of postharvest biocontrol research: is it time for a new paradigm? Postharvest Biol Technol 2009;52(2):137–45.
[2] Tian SP, Qin GZ, Li BQ. Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. Plant Mol Biol 2013;82(6):593–602.
[3] Statistics [Internet]. Rome: Food and Agriculture Organization of the United Nations; c2020 [cited 2019 Oct 1]. Available from: http://www.fao.org/statistics/en/.
[4] Tian SP, Torres R, Ballester AR, Li BQ, Vilanova L, González-Candelas L. Molecular aspects in pathogen–fruit interactions: virulence and resistance. Postharvest Biol Technol 2016;122:11–21.
[5] Klee HJ, Giovannoni JJ. Genetics and control of tomato fruit ripening and quality attributes. Annu Rev Genet 2011;45(1):41–59.
[6] Lü PT, Yu S, Zhu N, Chen YR, Zhou BY, Pan Y, et al. Genome encode analyses reveal the basis of convergent evolution of fleshy fruit ripening. Nat Plants 2018;4(10):784–91.
[7] Prusky DB. Pathogen quiescence in postharvest disease. Annu Rev Phytopathol 1996;34(1):413–34.
[8] Gillaspy G, Ben-David H, Gruissem W. Fruits: a developmental perspective. Plant Cell 1993;5(10):1439–51.
[9] Sisler EC, Serek M. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol Plant 2006;100(3):577–82.
[10] Liu RL, Xu Y, Li BQ, Qin GZ, Tian SP. The role of 1-methylcyclopropene in lipid peroxidation, anti-oxidant enzyme activities, and ethylene biosynthesis in 'Laiyang' pear (*Pyrus bretschneideri* Rehd.) during fruit ripening. J Hortic Sci Biotechnol 2015;90(2):210–6.
[11] Zhang ZQ, Tian SP, Zhu Z, Xu Y, Qin GZ. Effects of 1-methylcyclopropene (1-MCP) on ripening and resistance of jujube (*Zizyphus jujuba* cv. Huping) fruit against postharvest disease. LWT-Food Sci Technol 2012;45(1):13–9.
[12] Koukounaras A, Sfakiotakis E. Effect of 1-MCP prestorage treatment on ethylene and $CO_2$ production and quality of 'Hayward' kiwifruit during shelf-life after short, medium and long term cold storage. Postharvest Biol Technol 2007;46(2):174–80.
[13] Jiang Y, Joyce DC, Terry LA. 1-methylcyclopropene treatment affects strawberry fruit decay. Postharvest Biol Technol 2001;23(3):227–32.
[14] Liu RL, Wang YY, Qin GZ, Tian SP. Molecular basis of 1-methylcyclopropene regulating organic acid metabolism in apple fruit during storage. Postharvest Biol Technol 2016;117:57–63.
[15] Giovannoni JJ. Fruit ripening mutants yield insights into ripening control. Curr Opin Plant Biol 2007;10(3):283–9.
[16] Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, et al. A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (Rin) locus. Science 2002;296(5566):343–6.
[17] Ng TJ, Tigchelaar EC. Action of the non-ripening (*nor*) mutant on fruit ripening of tomato. J Am Soc Hortic Sci 1977;102:504–9.
[18] Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, et al. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 2006;38(8):948–52.
[19] Barry CS, Giovannoni JJ. Ripening in the tomato *Green-ripe* mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci USA 2006;103(20):7923–8.
[20] Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. The never ripe mutation blocks ethylene perception in tomato. Plant Cell 1994;6(4):521–30.
[21] Fujisawa M, Nakano T, Shima Y, Ito Y. A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. Plant Cell 2013;25(2):371–86.
[22] Qin GZ, Wang YY, Cao BH, Wang WH, Tian SP. Unraveling the regulatory network of the MADS box transcription factor RIN in fruit ripening. Plant J 2012;70(2):243–55.
[23] Wang YY, Wang WH, Cai JH, Zhang YR, Qin GZ, Tian SP. Tomato nuclear proteome reveals the involvement of specific $E_2$ ubiquitin-conjugating enzymes in fruit ripening. Genome Biol 2014;15(12):548–67.
[24] Zhong SL, Fei ZJ, Chen YR, Zheng Y, Huang MY, Vrebalov J, et al. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol 2013;31(2):154–9.
[25] Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Shima Y, Nakamura N, et al. Re-evaluation of the *rin* mutation and the role of RIN in the induction of tomato ripening. Nat Plants 2017;3(11):866–74.
[26] Li S, Xu H, Ju Z, Cao D, Zhu H, Fu D, et al. The RIN-MC fusion of MADS-box transcription factors has transcriptional activity and modulates expression of many ripening genes. Plant Physiol 2018;176(1):891–909.
[27] Wu J, Xu Z, Zhang Y, Chai L, Yi H, Deng X. An integrative analysis of the transcriptome and proteome of the pulp of a spontaneous late-ripening sweet orange mutant and its wild type improves our understanding of fruit ripening in citrus. J Exp Bot 2014;65(6):1651–71.
[28] Qin GZ, Zhu Z, Wang WH, Cai JH, Chen Y, Li L, et al. A tomato vacuolar invertase inhibitor mediates sucrose metabolism and influences fruit ripening. Plant Physiol 2016;172(3):1596–611.
[29] Cercós M, Soler G, Iglesias DJ, Gadea J, Forment J, Talón M. Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization. Plant Mol Biol 2006;62(4–5):513–27.
[30] Wang J, Liu J, Chen K, Li H, He J, Guan B, et al. Comparative transcriptome and proteome profiling of two citrus sinensis cultivars during fruit development and ripening. BMC Genomics 2017;18(1):984.
[31] Chen JW, Zhang SL, Zhang LC. Sugar transport, metabolism, accumulation and their regulation in fruits. J Plant Physiol Mol Biol 2004;30(1):1–10. Chinese.
[32] Arena ME, Zuleta A, Dyner L, Constenla D, Ceci L, Curvetto N. Berberis buxifolia fruit growth and ripening: evolution in carbohydrate and organic acid contents. Sci Hortic 2013;158:52–8.
[33] Jia H, Jiu S, Zhang C, Wang C, Tariq P, Liu Z, et al. Abscisic acid and sucrose regulate tomato and strawberry fruit ripening through the abscisic acid-stress-ripening transcription factor. Plant Biotechnol J 2016;14(10):2045–65.
[34] Gambetta GA, Matthews MA, Shaghasi TH, McElrone AJ, Castellarin SD. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. Planta 2010;232(1):219–34.
[35] Zhu A, Li W, Ye J, Sun X, Ding Y, Cheng Y, et al. Microarray expression profiling of postharvest Ponkan mandarin (*Citrus reticulata*) fruit under cold storage reveals regulatory gene candidates and implications on soluble sugars metabolism. J Integr Plant Biol 2011;53(5):358–74.
[36] Chai YM, Jia HF, Li CL, Dong QH, Shen YY. FaPYR1 is involved in strawberry fruit ripening. J Exp Bot 2011;62(14):5079–89.
[37] Jia HF, Chai YM, Li CL, Lu D, Luo JJ, Qin L, et al. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiol 2011;157(1):188–99.
[38] Etienne A, Génard M, Lobit P, Mbeguié-A-Mbéguié D, Bugaud C. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J Exp Bot 2013;64(6):1451–69.

[39] Yu K, Xu Q, Da X, Guo F, Ding Y, Deng X. Transcriptome changes during fruit development and ripening of sweet orange (*Citrus sinensis*). BMC Genomics 2012;13(1):10.
[40] Liu C, Long J, Zhu K, Liu L, Yang W, Zhang H, et al. Characterization of a citrus R2R3-MYB transcription factor that regulates the flavonol and hydroxycinnamic acid biosynthesis. Sci Rep 2016;6(1):25352.
[41] Huang D, Wang X, Tang Z, Yuan Y, Xu Y, He J, et al. Subfunctionalization of the *Ruby2-Ruby1* gene cluster during the domestication of citrus. Nat Plants 2018;4(11):930–41.
[42] An JP, Zhang XW, You CX, Bi SQ, Wang XF, Hao YJ. MdWRKY40 promotes wounding-induced anthocyanin biosynthesis in association with MdMYB1 and undergoes MdBT2-mediated degradation. New Phytol 2019;224 (1):380–95.
[43] An JP, Wang XF, Zhang XW, Xu HF, Bi SQ, You CX, et al. An apple MYB transcription factor regulates cold tolerance and anthocyanin accumulation and undergoes MIEL1-mediated degradation. Plant Biotechnol J 2020;18 (2):337–53.
[44] Mittler R. ROS are good. Trends Plant Sci 2017;22(1):11–9.
[45] Brennan T, Frenkel C. Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol 1977;59(3):411–6.
[46] Frenkel C, Eskin M. Ethylene evolution as related to changes in hydroperoxides in ripening tomato fruit. HortScience 1977;12:552–3.
[47] Warm E, Laties GG. Quantification of hydrogen peroxide in plant extracts by the chemiluminescence reaction with luminol. Phytochemistry 1982;21 (4):827–31.
[48] Qin GZ, Meng XH, Wang Q, Tian SP. Oxidative damage of mitochondrial proteins contributes to fruit senescence: a redox proteomics analysis. J Proteome Res 2009;8(5):2449–62.
[49] Meng XH, Han J, Wang Q, Tian SP. Changes in physiology and quality of peach fruits treated by methyl jasmonate under low temperature stress. Food Chem 2009;114(3):1028–35.
[50] Ding Y, Chang J, Ma Q, Chen L, Liu S, Jin S, et al. Network analysis of postharvest senescence process in citrus fruits revealed by transcriptomic and metabolomic profiling. Plant Physiol 2015;168(1):357–76.
[51] Wang Y, Ji DC, Chen T, Li BQ, Zhang ZQ, Qin GZ, et al. Production, signaling and scavenging mechanisms of reactive oxygen species in fruit-pathogen interactions. Int J Mol Sci 2019;20(12):2994.
[52] Chan ZL, Qin GZ, Xu XB, Li BQ, Tian SP. Proteome approach to characterize proteins induced by antagonist yeast and salicylic acid in peach fruit. J Proteome Res 2007;6(5):1677–88.
[53] Chan ZL, Wang Q, Xu XB, Meng XH, Qin GZ, Li BQ, et al. Functions of defense-related proteins and dehydrogenases in resistance response induced by salicylic acid in sweet cherry fruits at different maturity stages. Proteomics 2008;8(22):4791–807.
[54] Wang Q, Lai TF, Qin GZ, Tian SP. Response of jujube fruits to exogenous oxalic acid treatment based on proteomic analysis. Plant Cell Physiol 2009;50 (2):230–42.
[55] Zhang L, Kars I, Essenstam B, Liebrand TWH, Wagemakers L, Elberse J, et al. Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the arabidopsis receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1. Plant Physiol 2014;164(1):352–64.
[56] Weiberg A, Wang M, Lin FM, Zhao HW, Zhang ZH, Kaloshian I, et al. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 2013;342(6154):118–23.
[57] Cai Q, Qiao LL, Wang M, He BY, Lin FM, Palmquist J, et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science 2018;360(6393):1126–9.
[58] An B, Li BQ, Li H, Zhang ZQ, Qin GZ, Tian SP. Aquaporin8 regulates cellular development and reactive oxygen species production, a critical component of virulence in *Botrytis cinerea*. New Phytol 2015;209(4):1668–80.
[59] Li BQ, Peng HM, Tian SP. Attachment capability of antagonistic yeast *Rhodotorula glutinis* to *Botrytis cinerea* contributes to biocontrol efficacy. Front Microbiol 2016;7:601.
[60] Takemoto D, Tanaka A, Scott B. NADPH oxidases in fungi: diverse roles of reactive oxygen species in fungal cellular differentiation. Fungal Genet Biol 2007;44(11):1065–76.
[61] Segmüller N, Kokkelink L, Giesbert S, Odinius D, van Kan JA, Tudzynski P. NADPH oxidases are involved in differentiation and pathogenicity in *Botrytis cinerea*. Mol Plant Microbe Interact 2008;21(6):808–19.
[62] Siegmund U, Heller J, van Kan JAL, Tudzynski P. The NADPH oxidase complexes in *Botrytis cinerea*: evidence for a close association with the ER and the tetraspanin Pls1. PLoS ONE 2013;8(2):e55879.
[63] Minz Dub A, Kokkelink L, Tudzynski B, Tudzynski P, Sharon A. Involvement of *Botrytis cinerea* small GTPases BcRAS1 and BcRAC in differentiation, virulence, and the cell cycle. Eukaryot Cell 2013;12(12):1609–18.
[64] Li H, Zhang ZQ, He C, Qin GZ, Tian SP. Comparative proteomics reveals the potential targets of BcNoxR, a putative regulatory subunit of NADPH oxidase of *Botrytis cinerea*. Mol Plant Microbe Interact 2016;29(12):990–1003.
[65] Gourgues M, Brunet-Simon A, Lebrun MH, Levis C. The tetraspanin BcPls1 is required for appressorium-mediated penetration of *Botrytis cinerea* into host plant leaves. Mol Microbiol 2004;51(3):619–29.
[66] Julca I, Droby S, Sela N, Marcet-Houben M, Gabaldón T. Contrasting genomic diversity in two closely related postharvest pathogens: *Penicillium digitatum* and *Penicillium expansum*. Genome Biol Evol 2015;8(1):218–27.
[67] Li BQ, Zong YY, Du ZL, Chen Y, Zhang ZQ, Qin GZ, et al. Genomic characterization reveals insights into patulin biosynthesis and pathogenicity in *Penicillium* species. Mol Plant Microbe Interact 2015;28 (6):635–47.
[68] Chen Y, Li BQ, Xu XD, Zhang ZQ, Tian SP. The pH-responsive PacC transcription factor plays pivotal roles in virulence and patulin biosynthesis in *Penicillium expansum*. Environ Microbiol 2018;20(11):4063–78.
[69] Jurick WM, Peng H, Beard HS, Garrett WM, Lichtner FJ, Luciano-Rosario D, et al. Blistering1 modulates *Penicillium expansum* virulence via vesicle-mediated protein secretion. Mol Cell Proteomics 2020;19(2):344–61.
[70] Tian SP. Microbial control of postharvest diseases of fruits and vegetables: current concepts and future outlook. In: Ray RC, Ward OP, editors. Microbial biotechnology in horticulture. Enfield: Science Publishers; 2006. p. 163–202.
[71] Wisniewski M, Wilson C, Droby S, Chalutz E, El Ghaouth A, Stevens C. Postharvest biocontrol: new concepts and applications. In: Vincent C, Goettel MS, Lazarovits G, editors. Biological control: a global perspective. Cambridge: CAB International; 2007. p. 262–73.
[72] Sharma RR, Singh D, Singh R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review. Biol Control 2009;50 (3):205–21.
[73] Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. Int J Food Microbiol 2013;167(2):153–60.
[74] Spadaro D, Droby S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends Food Sci Technol 2016;47:39–49.
[75] Yu T, Li HY, Zheng XD. Synergistic effect of chitosan and *Cryptococcus laurentii* on inhibition of *Penicillium expansum* infections. Int J Food Microbiol 2007;114(3):261–6.
[76] Yu C, Zhou T, Sheng K, Zeng L, Ye C, Yu T, et al. Effect of pyrimethanil on *Cryptococcus laurentii, Rhodosporidium paludigenum*, and *Rhodotorula glutinis* biocontrol of *Penicillium expansum* infection in pear fruit. Int J Food Microbiol 2013;164(2–3):155–60.
[77] Zhang HY, Ma LC, Turner M, Xu HX, Zheng XD, Dong Y, et al. Salicylic acid enhances biocontrol efficacy of *Rhodotorula glutinis* against postharvest *Rhizopus* rot of strawberries and the possible mechanisms involved. Food Chem 2010;122(3):577–83.
[78] Zhang D, Spadaro D, Garibaldi A, Gullino ML. Potential biocontrol activity of a strain of *Pichia guilliermondii* against grey mold of apples and its possible modes of action. Biol Control 2011;57(3):193–201.
[79] Lahlali R, Hamadi Y, Drider R, Misson C, El Guilli M, Jijakli MH. Control of citrus blue mold by the antagonist yeast *Pichia guilliermondii* Z1: compatibility with commercial fruit waxes and putative mechanisms of action. Food Control 2014;45:8–15.
[80] Yan F, Xu S, Chen Y, Zheng X. Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in cherry tomato fruit. Postharvest Biol Technol 2014;97:32–5.
[81] Zhang ZQ, Qin GZ, Li BQ, Tian SP. Effect of cinnamic acid for controlling gray mold on table grape and its possible mechanisms of action. Curr Microbiol 2015;71(3):396–402.
[82] Cai JH, Chen J, Lu GB, Zhao YM, Tian SP, Qin GZ. Control of brown rot on jujube and peach fruits by trisodium phosphate. Postharvest Biol Technol 2015;99:93–8.
[83] Ji DC, Chen T, Ma DY, Liu JL, Xu Y, Tian SP. Inhibitory effects of methyl thujate on mycelial growth of *Botrytis cinerea* and possible mechanisms. Postharvest Biol Technol 2018;142:46–54.
[84] Meng XH, Li BQ, Liu J, Tian SP. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. Food Chem 2008;106(2):501–8.
[85] He C, Zhang ZQ, Li BQ, Xu Y, Tian SP. Effect of natamycin on *Botrytis cinerea* and *Penicillium expansum*—postharvest pathogens of grape berries and jujube fruit. Postharvest Biol Technol 2019;151:134–41.
[86] Ma DY, Ji DC, Zhang ZQ, Li BQ, Qin GZ, Xu Y, et al. Efficacy of rapamycin in modulating autophagic activity of *Botrytis cinerea* for controlling gray mold. Postharvest Biol Technol 2019;150:158–65.
[87] Wiemann P, Guo CJ, Palmer JM, Sekonyela R, Wang CCC, Keller NP. Prototype of an intertwined secondary-metabolite supercluster. Proc Natl Acad Sci USA 2013;110(42):17065–70.
[88] Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003;16(3):497–516.
[89] Qin GZ, Tian SP, Chan ZL, Li BQ. Crucial role of antioxidant proteins and hydrolytic enzymes in pathogenicity of *Penicillium expansum*: analysis based on proteomics approach. Mol Cell Proteomics 2007;6(3):425–38.
[90] Piqué E, Vargas-Murga L, Gómez-Catalán J, de Lapuente J, Llobet JM. Occurrence of patulin in organic and conventional apple-based food marketed in Catalonia and exposure assessment. Food Chem Toxicol 2013;60:199–204.
[91] Chalmers I, Clarke M. Commentary: the 1944 patulin trial: the first properly controlled multicentre trial conducted under the aegis of the British Medical Research Council. Int J Epidemiol 2004;33(2):253–60.
[92] Puel O, Galtier P, Oswald IP. Biosynthesis and toxicological effects of patulin. Toxins 2010;2(4):613–31.
[93] Artigot MP, Loiseau N, Laffitte J, Mas-Reguieg L, Tadrist S, Oswald IP, et al. Molecular cloning and functional characterization of two CYP619 cytochrome P450s involved in biosynthesis of patulin in *Aspergillus clavatus*. Microbiology 2009;155(Pt 5):1738–47.

[94] Banani H, Marcet-Houben M, Ballester AR, Abbruscato P, González-Candelas L, Gabaldón T, et al. Genome sequencing and secondary metabolism of the postharvest pathogen *Penicillium griseofulvum*. BMC Genomics 2016;17(1):19.
[95] Tannous J, El Khoury R, Snini SP, Lippi Y, El Khoury A, Atoui A, et al. Sequencing, physical organization and kinetic expression of the patulin biosynthetic gene cluster from *Penicillium expansum*. Int J Food Microbiol 2014;189:51–60.
[96] Ballester AR, Marcet-Houben M, Levin E, Sela N, Selma-Lázaro C, Carmona L, et al. Genome, transcriptome, and functional analyses of *Penicillium expansum* provide new insights into secondary metabolism and pathogenicity. Mol Plant Microbe Interact 2015;28(3):232–48.
[97] Li BQ, Zong YY, Du ZL, Chen Y, Zhang ZQ, Qin GZ, et al. Genomic characterization reveals insights into patulin biosynthesis and pathogenicity in *Penicillium* species. Mol Plant Microbe Interact 2015;28(6):635–47.
[98] Yu JJ, Chang PK, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, et al. Clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol 2004;70(3):1253–62.
[99] Li BQ, Chen Y, Zong YY, Shang YJ, Zhang ZQ, Xu XD, et al. Dissection of patulin biosynthesis, spatial control and regulation mechanism in *Penicillium expansum*. Environ Microbiol 2019;21(3):1124–39.
[100] Forouzan S, Madadlou A. Incidence of patulin in apple juices produced in west Azerbayjan Province, Iran. J Agric Sci Technol 2014;16:1613–22.
[101] Moss MO, Long MT. Fate of patulin in the presence of the yeast *Saccharomyces cerevisiae*. Food Addit Contam 2002;19(4):387–99.
[102] Stinson EE, Osman SF, Huhtanen CN, Billis DD. Disappearance of patulin during alcoholic fermentation of apple juice. Appl Environ Microbiol 1978;36(4):620–2.
[103] Ferruz E, Loran S, Herrera M, Gimenez I, Bervis N, Barcena C, et al. Inhibition of *Fusarium* growth and mycotoxin production in culture medium and in maize kernels by natural phenolic acids. J Food Protect 2016;79(10):1753–8.