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Review

Augmentation of biocontrol agents with physical methods against postharvest diseases of fruits and vegetables

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ABSTRACT

Background: The application of physical (thermal and non-thermal) treatments in combination with biocontrol agents for the control of postharvest fungi has achieved significant research attention. In order to make combined nonchemical agents commercially suitable for postharvest treatment of other commodities, there is the need to study their individual effects and then integrated effects to present them as economically viable, resilient and persistent.

Scope and approach: In this article, various physical treatment methods (thermal and non-thermal) have been used to enhance the bioefficacy of microbial agents against postharvest diseases of fruits and the possible mode of action were reviewed. Additionally, the interrelationship between fungal virulence, host response and environmental factors that influence infection rate and production of mycotoxin has also been highlighted.

Key findings and conclusions: Physical treatments act as disinfectants of surfaces of produce prior to application of antagonistic yeasts that provide persistent protective action over an extensive period. Physical methods by heat treatment tends to seal or cure openings on the produce in order to limit the sites for pathogen penetration and restriction of secondary infections by biofilm formation after application of antagonistic yeasts or bioactive compounds. Thus, this phenomenon slow down changes in fruits respiration and metabolic activity. Heat has the potential to up-regulate proteins in fruits, which correlates with defense response and redox metabolism; consequently, demonstrates a physiological adaptation to environmental stress. From literature, there is limited information on the effect of ohmic heat method combined with antagonistic on decay causing pathogens and shelf life of fruits. Furthermore, the employment of novel tools available in molecular biology will enable in-depth explanation of other physiological and biochemical pathways on which the treatment effects are supported.

1. Introduction

The agroalimentary industry has been focused not only on addressing the growing demand for food security but also on the health and safety of consumers, which has expanded considerably the *status quo* of innovative postharvest diseases control strategies. Currently, fruits and vegetables losses caused by postharvest diseases are estimated between 35 and 55% of the total production, with significant differences of more than 55% among the geo-economic areas such as in developing countries (Sanzani, Reverberi, & Geisen, 2016). As recently reported in their review paper, significant proportions of the losses are attributed to damage caused by fungal species, and associated mycotoxin-contamination (Mahunu, Zhang, Yang, Li, & Zheng, 2015). Several methods have been proposed for the control of these diseases and some proven to be promising. Thus, there is evidence that investing in postharvest disease control after harvest greatly surpasses the outcome of increasing food production to make up for the losses. In spite of this, only 5% of the investment into food production is allocated to protection of food at postharvest stage (Kader, 2012). Wilson (2013) recently emphasized that advanced food storage technologies can

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save nearly 50% of annual food losses.

For many decades synthetic fungicides are being applied immediately before or after harvest to manage postharvest deterioration of fruit (Lima et al., 2011). According to Pinela and Ferreira (2017), chemical treatments do not provide an efficient reduction of microbial load, since they are perceived negatively by the consumers, dangerous for human health, and harmful to the environment. The restriction on their application is also because of possible occurrence of fungicide-resistant strains of fungal pathogens. To overcome these challenges, several alternative or integrative approaches are becoming increasingly imperative in controlling postharvest fungal disease and at the same time maintaining a high level of quality. These include biological control agents, natural compounds, decontaminating agents, physical methods, and their combinations (Feliziani & Romanazzi, 2016).

Among them, biocontrol by antagonistic microorganisms (including yeasts, yeast-like fungi and bacteria) are becoming more accepted as consumers demand for products with a very limited number of residues continue to increase. Besides, other approaches such as physical treatment, as a nonchemical technique have gained enormous interest in worldwide to control various postharvest diseases due to the total absence of residues in the final product and minimal environmental impact. However, in recent times the combination of two or more treatments rather than single treatment has been recommended for application as they provide sufficient control of decay causing fungi (Lima, De Curtis, & De Cicco, 2008).

This review discusses the most relevant scientific works concerning biological control (biocontrol), physical methods and their combined treatment effects on perishable fruits and vegetables published in the last ten years with the aim of directing future investigations and upscaling. Other aspects of the effects of physical or combined treatment on storage quality and gene expression was included in the discussion.

2. Biological control

According to Pal and Gardener (2006), biocontrol (BC) deals with the purposeful utilization of introduced or resident living organisms, apart from the disease resistant host plant, to suppress the activities and populations of one or more plant pathogens or reproduction of one organism using another organism. Similarly, Vinale, et al. (2008) considered biological control as the employment of beneficial organisms, their genes, and/or products, such as metabolites, so as to reduce the negative effects of plant pathogens and promote positive responses in the plant. This implies that the organism that contributes to the suppression of the pathogen is referred to as the biological control agent (BCA). Usually, BC has also been related to the use natural products extracted from various sources. Formulations of BCAs may vary from very simple combination of natural ingredients with specific activities to complex combinations with multiple effects on the host as well as the target pest or pathogen. The natural mixtures may imitate the activities of living organisms, whereas the non-living inputs may be more appropriately considered as biopesticides or biofertilizers, depending on the primary benefit they provide to the host plant (Nega, 2014).

The fundamental model of biocontrol is reducing disease occurrence or its severity through direct or indirect manipulation of microorganisms (Shurtleff & Averre, 1997) and/or the host environment (Prusky, Barad, Luria, & Ment, 2014). Some unique qualities of microbial organisms make them very useful competent of the novel approach to postharvest diseases control. According to Harman, Howell, Viterbo, Chet, and Lorito (2004) these qualities of microbial organisms include their ability [1] to compete for space and nutrient that allow rapid establishment within the stable microbial communities in the host plant, [2] to control of pathogenic and competitive/deleterious microflora through diverse mechanisms; [3] to improve plant health, and [4] to stimulate root growth (Harman et al., 2004).

It was also reported that yeasts are tolerant to extreme environmental conditions during pre- and post-harvest, the unique adaptation to the fruit microenvironment, their ability grow rapidly on less costly medium and as well as easy to produce in large quantities (Spadaro, Ciavorella, Dianpeng, Garibaldi, & Gullino, 2010). Other promising qualities of yeasts is based on the fact that they do not produce allergenic spores or mycotoxins, compared to filamentous fungi, and they have simple nutritional requirements that permit them to colonize dry surfaces for extended period. Their competition for nutrients such as amino acids, sugars and $Fe_{3+,}$ as an important mechanism enable yeasts to compete for nutrients against other antagonists (Bencheqroun et al., 2007; Saravanakumar, Ciavorella, Spadaro, Garibaldi, & Gullino, 2008; Sipiczki, 2006). Additionally, modes of action such as mycoparasitism, induced resistance and the production of lytic enzymes viz., β-1, 3-glucanase and chitinase exhibited by yeasts have been reported (Ippolito & Nigro, 2000; Saligkarias, Gravanis, & Epton, 2002; Yu, Wang, Yin, Wang, & Zheng, 2008).

In another facet, the microorganisms that are harnessed for biocontrol of diseases have a wide range of modes of action, categorized into two broad areas (direct and indirect effect). First, direct microbial effect is where the microbial antagonists that occupy the same ecological niche as the target pathogen and interact directly with it. Here, the mechanisms of interaction include parasitism, competition for space, nutrients or ‘chemical warfare’ using antibiotics or other secondary metabolites that harm the target pathogen. Secondly, an indirect effect is where the biocontrol agent induces a resistance response in the host that gives it protection against plant pathogens. Actually, the ‘inducer’ for this form of control may utilize a particular strain of the plant pathogen that has low virulence, a different species of microorganism or a natural product.

Some biological control products have been developed and commercialized. These include formulations of Biosave™ based on *Pseudomonas syringae* Van Hall (Janisiewicz & Peterson, 2004), Shemer™ based on *Metschnikowia fructicola* (Droby, Wisniewski, Macarisin, & Wilson, 2009), Nexy based on another strain of *C. oleophila* and BoniProtect™ was also developed based on two antagonistic strains of *Aureobasidium pullulans*.

3. Physical methods used in harvested fruits and vegetables

In recent years, physical methods has gained research attention in the control of several postharvest diseases since they are nonchemical chemical techniques, with minimal health and environmental impact (Usall, Ippolito, Sisquella, & Neri, 2016). They have been used to control or render plant pathogens harmless but its application to control decay of fresh fruits and vegetables vary from other uses. In general, physical methods have been categorized into two: thermal and non-thermal.

Thermal treatment is the application of heat as a short treatment before cold storage (Escribano & Mitcham, 2014). Various heat treatment methods that have been reported include microwave (Das, Shah, & Kumar, 2014; Sisquella, Vinas, Teixidó, Picouet, & Usall, 2013; Sisquella Sanagustín et al., 2014), hot water dip, hot water rinsing and brushing, vapor, hot air and curing (Fallik, 2004; Huan et al., 2017). Heat is a highly effective method of controlling microorganisms. Mostly, various heat treatments use temperatures above 40 °C for control of postharvest pathogens; since fruits and vegetables normally tolerate temperatures of 50–60 °C for