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Combining an antagonistic yeast with harpin treatment to control postharvest decay of kiwifruit

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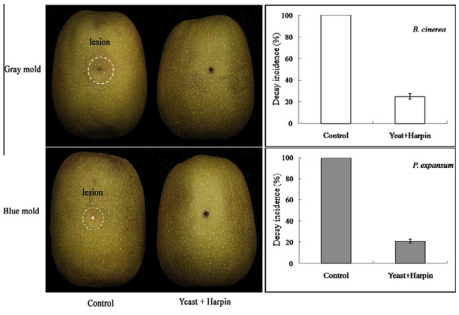
HIGHLIGHTS

- *C. diversa* or harpin effectively controlled postharvest decay of kiwifruit.
- Harpin enhanced biocontrol efficacy of *C. diversa* against postharvest decay.
- *C. diversa* and harpin alone or in combination induced kiwi defense response.
- An integrated strategy of *C. diversa* and harpin treatment is a promising approach.

GRAPHICAL ABSTRACT



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ABSTRACT

Integrated management, utilizing different non-chemical methods, is an approach to controlling postharvest losses that is being actively investigated. In the present study, the use of an antagonistic yeast, *Candida diversa*, combined with harpin (a hypersensitive response elicitor) treatment was evaluated for their ability to prevent infection of kiwifruit after they were artificially inoculated with *Botrytis cinerea* or *Penicillium expansum*. Natural infection of treated fruit was also assessed. As a standalone treatment, *C. diversa* or harpin significantly reduced gray (*B. cinerea*) and blue mold (*P. expansum*) infections on kiwifruit, relative to the untreated control fruit, and also reduced the level of natural infection. The combination of *C. diversa* and harpin treatment, however, provided a superior level of control on fruit, relative to either treatment alone. Harpin did not negatively impact *C. diversa* growth in kiwifruit wounds. Treatment of kiwifruit with *C. diversa* and harpin alone or in combination also induced the activity of enzymes involved in defense response, such as polyphenol oxidase, peroxidase and superoxide dismutase. Harpin alone or in combination with *C. diversa* enhanced accumulation of lignin content in kiwifruit as well. The mode of action by which harpin enhanced biocontrol efficacy of *C. diversa* may be partially attributed to the elicitation of defense response in kiwifruit. Integrating the use of antagonistic yeast with harpin treatment has potential as an effective method for the control of postharvest decay in kiwifruit.

1. Introduction

Kiwifruit has long been called ‘the king of fruits’ due to its high vitamin C content and balanced composition of minerals, dietary fiber, and other metabolites beneficial to human health (Huang

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et al., 2013; Stonehouse et al., 2013). Postharvest decay of kiwifruit due to infections by fungal pathogens, however, results in significant losses. Gray mold caused by *Botrytis cinerea* is the most important postharvest disease of kiwifruit (Michailides and Elmer, 2000; Minas et al., 2010), while blue mold caused by *Penicillium expansum* is another disease on kiwifruit (Neri et al., 2010; Wang and Buta, 2003). Synthetic chemical fungicides are still the main method used to control postharvest decay of kiwifruit (Bardas et al., 2010). Development of resistant biotypes of the pathogens, however, as well as public concern over the potential impact of fungicide residues on human health and environment, have created great interest in the development of alternative methods for the control of postharvest diseases (Sivakumar and Bautista-Baños, 2014; Terry and Joyce, 2004; Wilson and Wisniewski, 1989; Wisniewski and Wilson, 1992).

Among eco-friendly approaches, biological control utilizing antagonistic yeasts has been reported to be effective in managing postharvest decay of a variety of fruits (Droby et al., 2009; Liu et al., 2013; Sharma et al., 2009; Spadaro and Gullino, 2004). Many yeasts in the genus *Candida*, including *Candida oleophila*, *Candida sake*, *Candida saitoana*, and *Candida diversa*, have been reported as effective biocontrol agents for the control of postharvest diseases of apple, pear, grapefruit, table grape and sweet cherry (Droby et al., 2002; Lahlali and Jijakli, 2009; Nunes et al., 2001; Raspor et al., 2010; Schena et al., 2005). In addition to the use of antagonists, induction of disease resistance by elicitor in postharvest horticultural crops is another potential alternative to synthetic chemical fungicides (Terry and Joyce, 2004; Tian et al., 2006). As a hypersensitive response (HR) elicitor, harpin is an acidic, heat-stable, glycinerich protein, encoded by the *hrp* gene of *Erwinia amylovora* (Wei et al., 1992). Harpin could elicit HR in Arabidopsis and tobacco leaves (Ger et al., 2014; Li et al., 2013), as well as induce defense response/improve shelf life of fruits and vegetables like apple (de Capdeville et al., 2003), peach (Taylor, 2006), melon (Yang et al., 2005), tomato (Akbudak et al., 2006), jujube (Li et al., 2012), lettuce (Fonseca et al., 2009) and pepper (Tezcan et al., 2013).

While biological treatment using antagonistic yeast or elicitation treatment using harpin has demonstrated to be effective in reducing postharvest decay, an integrated management approach will most likely be needed to provide a viable alternative to chemical fungicides that can be used effectively under a wide array of environmental conditions (Smilanick, 2008). Thus far, little information has been provided on how harpin treatment enhances the biocontrol efficacy of antagonistic yeasts against postharvest decay of kiwifruit. The objective of the present study was to evaluate the effects of harpin treatment and the yeast, *C. diversa*, isolated from kiwifruit, applied separately or in combination, on the control of postharvest decay of kiwifruit. The effects of harpin on the population dynamics of *C. diversa in vivo*, and on the defense response of kiwifruit were assessed as well.

2. Materials and methods

2.1. Antagonistic yeast

The yeast, *C. diversa*, was isolated from the surface of kiwifruit by ourselves, and identified by its general morphology and sequence of the ITS region of ribosomal DNA according to Leaw et al. (2006). *C. diversa* was cultured in 100 ml of yeast peptone dextrose (YPD) broth (10 g of yeast extract, 20 g of peptone and 20 g of dextrose in 1 l of water) in 500-ml conical flasks inoculated at an initial concentration of 10^5 cells/ml. Yeast cultures were incubated at 25 °C on a rotary shaker at 200 rpm for 48 h. Prior to use in the biocontrol assays, yeast cells were pelleted at 5000 g for 3 min and washed three times with sterile-distilled water in order to remove residual medium. The cell concentration was determined using a hemocytometer and adjusted to 5×10^7 cells/ml with sterile distilled water.

2.2. Fungal pathogens

The fungal pathogens, *B. cinerea* and *P. expansum*, were isolated from infected kiwifruit and maintained on potato dextrose agar (PDA) at 4 °C. To reactivate the culture and verify their pathogenicity, the pathogens were inoculated into wounded kiwifruit and re-isolated onto PDA after infection was established. Spore suspensions of each of the pathogens were obtained from 2-week-old PDA cultures at 25 °C, and spore concentration was determined using a hemocytometer and adjusted to 10^4 spores/ml with sterile distilled water prior to use.

2.3. Fruit

Kiwifruits (*Actinidia chinensis* cv. Hongyang) were harvested at commercial maturity (average quality values: 7.2 °Bx of brix, 62 N of firmness and 6.8 kg per 100 fruits). Fruits without wounds or rot were selected based on uniformity of size, disinfected with 2% (v/v) sodium hypochlorite for 2 min, rinsed with tap water, and air-dried.

2.4. Effect of harpin treatment on control of B. cinerea and P. expansum on kiwifruit

A range of harpin (Messenger®, Eden Bioscience Co., USA) treatments at the concentrations of 0, 60, 90, 120 mg/l were applied on kiwifruit in order to ensure that an appropriate, non-injurious dosage could be identified. The range of dosages was based on the previous studies (de Capdeville et al., 2003; Yang et al., 2005) and our own preliminary experiments.

Kiwifruits were randomly grouped into four lots. Three lots of fruit were immersed in harpin solution at 60, 90 and 120 mg/l for 10 min, respectively, while the fourth lot of fruit immersed in water served as a control. After 24 h, two wounds (3 mm deep × 3 mm wide) were made with a sterile nail on the opposite sides at the equator of each fruit. Five microliter of a spore suspension of either *B. cinerea* or *P. expansum* (1×10^4 spores/ml) was then inoculated into each wound. Treated fruits were placed in a covered plastic food tray, and each tray was enclosed within a polyethylene bag and stored at 20 °C. Decay incidence and lesion diameter of gray (*B. cinerea*) and blue mold (*P. expansum*) on each fruit was determined after 4 days. Decay incidence represents the percentage of infected wounds, while lesion diameter was measured only on those wounds that were infected. Each treatment contained three replicates of 20 fruits each and the experiment was repeated three times.

2.5. Effect of harpin treatment in combination with C. diversa on infection and development of B. cinerea and P. expansum on kiwifruit

Following disinfection of the fruit as previously described, kiwifruits were divided into four groups as follows:

Group I (harpin treatment): fruits were immersed in harpin solution at 90 mg/l for 10 min, then air-dried and wounded as described above (two wounds on the opposite sides at the equator of each fruit) and mock-inoculated with 5 µl of sterile water into each wound.

Group II (yeast treatment): fruits were not treated with harpin, but wounded and inoculated by pipetting 5 µl of *C. diversa* (5×10^7 cells/ml) into each wound.