Antifungal activity of *Lactobacillus plantarum* C10 against *Trichothecium roseum* and its application in promotion of defense responses in muskmelon (*Cucumis melo* L.) fruit

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Abstract The antifungal effect of *Lactobacillus plantarum* C10 on pink rot caused by *Trichothecium roseum* and its application in muskmelon fruit were investigated. Cell-free supernatant (CFS) produced by *Lactobacillus plantarum* C10 strongly inhibited the growth of *T. roseum* and seriously damaged the structures of spores and mycelia of *T. roseum*. Acid compounds produced by *Lb. plantarum* C10 were the major antifungal substances and exhibited a narrow pH range from 3.5 to 6.5. Application of the CFS on muskmelon fruit reduced the contamination zone of *T. roseum* by enhancing the activities of defensive enzymes (phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase) and promoting the accumulation of phenolics and flavonoids. These results suggested that *Lb. plantarum* C10 could be used as a biocontrol agent to control pink rot caused by *T. roseum* in muskmelon fruit.

Keywords *Lactobacillus plantarum* C10 · Antifungal activity · *Trichothecium roseum* · Acid compounds · Defensive enzymes · Muskmelon fruit

Introduction

Muskmelon (*Cucumis melo* L.) is major crops with high economic value due to their unique flavor and rich nutrition, which is widely grown in Northeast and Northwestern of China (Deng et al. 2015). However, it is quite perishable and susceptible to postharvest rot caused by phytopathogenic fungi (Yang et al. 2009). Pink rot caused by *Trichothecium roseum* is one of the most principal postharvest diseases of muskmelon in China (Ge et al. 2015). This disease is frequently controlled by the application of synthetic fungicides (Yang et al. 2009). Although synthetic fungicides have greatly extended the shelf life of field crops, postharvest losses still high at 50% in developing countries, with molds causing more than 70% of losses in fruits and vegetable storage (Frankova et al. 2016). Moreover, the continuous use of fungicides has attracted public concern on fungicide residues, development of fungicides resistance in pathogens, and potential harmful effects on human health and environmental safety (Tripathi and Dubey 2004; Yang et al. 2009). Therefore, alternative strategies to fungicides, that will be both safe and eco-friendly, have been widely sought (Pawlowska et al. 2012; Terry 2004; Tsuda et al. 2016).

Bio-preservation has emerged as an excellent candidate, which refers to the use of microorganisms and/or their metabolites to extend the shelf life and enhance the safety of foods (Cheong et al. 2014; Galvez et al. 2010; Plaza et al. 2016). Lactic acid bacteria (LAB), classed as generally regarded as safe (GRAS), have been used as bio-preservation organisms in foods for inhibiting the growth of fungi through the production of organic acids, fatty acids (Gerez et al. 2013), hydrogen peroxide, reuterin and bacteriocins (Yang and Chang 2010). Previous studies have exhibited the effectiveness of LAB in protecting different

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fruit species against various fungal diseases (Ghosh et al. 2015; Lan et al. 2012; Wang et al. 2013a). Lan et al. (2012) found that the antifungal strain *Weissella cibaria* 861006 inhibit the growth of *Penicillium oxalicum* on the surface of grapes. Moreover, *Pediococcus pentosaceus* 54 is shown to have protective properties against *Penicillium expansum* spoilage when applied in plum, pear and grape models (Crowley et al. 2013b). To our knowledge, there have been no reports on the antifungal effect of LAB against pink rot caused by *Trichothecium roseum* and on whether LAB influence on the activity of defense-related enzymes of muskmelon fruit.

The aims of this study were: (1) to obtain LAB with antifungal activity against *T. roseum*, (2) to analyze the major antifungal substances produced by LAB, and (3) to investigate the effect of cell-free supernatant produced by LAB on the activity of defense-related enzymes in muskmelon fruit.

Materials and methods

Microorganisms and culture conditions

Four LAB species, *Lactobacillus plantarum* C10, *Lb. rhamnosus* RH-11, *Lb. helveticus* LH-9 and *Lb. sakei* DL11 were provided from Microbiology Laboratory Culture Collection. All LAB strains were grown in Mann Rogosa Sharpe (MRS, Aoboxing, Beijing, China) broth at 37 °C for 24 h. Cell-free supernatant (CFS) produced by LAB was obtained by centrifugation at 6000g for 15 min at 4 °C and sterile filtration (0.45 µm, Millipore). Target bacteria *T. roseum* isolated from decayed muskmelon fruit, was grown on potato dextrose agar (PDA, Aoboxing, Beijing, China) at 28 °C for 7 days. The spores were collected in sterile Tween 80 at 0.05% (v/v) and counted at the microscope in a haemocytometer chamber, which concentration was used to adjust to 1×10^6 spores/ml.

Antifungal activity assays

Antifungal activity assay was performed according to the method as described by Wang et al. (2011). PDA (25 ml) containing CFS (2.5 ml) was poured into sterile plate. After solidification, this plate was inoculated with agar discs of the *T. roseum* (7 mm) at the center and incubated at 28 °C for 2–7 days. Each dish was diametrically monitored in perpendicular directions until the fungi growth in the control plate was almost complete. The inhibitory rate (I) was calculated as follow: $I (\%) = [(C - T) / (C - C_0)] \times 100$. C means the diameter of mycelia growth in control group (mm), T means the diameter of mycelia growth in treated group (mm), and C_0 means the diameter

of the target fungi agar discs (7 mm). Control plates containing media mixed with sterile water (10%, v/v) were inoculated.

Effect of CFS on the spore and mycelial morphology of *T. roseum*

MRS agar (15 ml) was poured into a petri dish. CFS was inoculated as two 3 cm long lines and incubated at 37 °C for 72 h. This plate was then overlaid with PDA (15 ml) containing 1×10^6 spores/ml and incubated at 28 °C for 6 days. The mycelia were harvested from the cultures grown on PDA. Spores were prepared by washing with 0.85% NaCl containing Tween 80 (0.1% v/v) and then centrifuged at 6000g for 15 min. The sediments were collected for SEM.

The mycelia and spores were rinsed with phosphate buffer (0.1 mol/l, pH 7.4), and then fixed with 2.5% glutaraldehyde at 4 °C for 24 h. The sediments were dehydrated by a graded series of ethanol (50, 70, 80, 90, 95 and 100%) for 20 min at each step. After dehydration, these samples were dried with vacuum freezing dryer (Free Zone 2.5 L, Labconco, USA). Finally, all samples were coated with gold–palladium and observed using S-4800 SEM (Hitachi, Japan).

Quantification of organic acids and phenyllactic acid (PLA)

Lactic acid, acetic acid and propionic acid in the CFS were quantified by Agilent 6980N gas chromatograph system (Agilent, USA) equipped with CNW CD-ACID WAX column (30 m $\times$ 0.25 mm $\times$ 0.25 µm). The column temperature programme was: initial temperature of 110 °C, then raised to 150 °C at 10 °C/min and temperature held for 5 min, and finally increased to 230 °C at 10 °C/min, and held for 15 min. The injector temperature and FID detector temperature were 280 and 300 °C, respectively. The flow rate of hydrogen and nitrogen carrier gas was 30 ml/min, and the flow rate of air was 300 ml/min. One microliter sample was injected in the split mode (15:1).

PLA was determined by Agilent 6980 N gas chromatograph system (Agilent, USA) equipped with RTX-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 µm). The column temperature was initially held at 180 °C for 20 min, and then raised to 280 °C at 20 °C/min, held for 10 min. The injector temperature and FID detector temperature were 280 and 300 °C, respectively. The flow rate of hydrogen and nitrogen carrier gas was 30 ml/min, and the flow rate of air was 300 ml/min. One microliter sample was injected in the split mode (20:1).

Determination of the antifungal activities of acids mixtures

Acids mixtures included lactic acid (Sigma L6661, USA), acetic acid (Sigma 695092, USA), propionic acid (Sigma 402907, USA) and PLA (Sigma P7251, USA). The individual acid and acids mixtures were prepared according to the data obtained after quantification by gas chromatograph analysis. The pH of all samples assayed (CFS, individual acids and acids mixtures) was adjusted to pH 4.0. The antifungal activities of all samples were assayed using previously described method.

Effect of pH on antifungal activity of CFS

Aliquots of CFS were adjusted to pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 respectively, by adding the appropriate volumes of 4 N HCl and 4 N NaOH. The antifungal activity was tested by previously described method.

Effect of LAB on muskmelon fruit decay

Muskmelon fruit was selected for uniformity of size, absence of defects, and washed with running water. After drying in air, the fruit were disinfected with 2% (v/v) sodium hypochlorite for 5 min, and washed with sterile water, air-dried, then sterilized with 70% ethanol. Three wounds (3 mm deep $\times$ 3 mm wide) were punched in each fruit using a sterile needle, and then 20 μ l of CFS and a ring fungal mycelium were inoculated in each wound and incubated at 25 °C.

Effect of CFS on induction of defense-related enzyme activities

For enzyme extraction, approximate 1.5 g of sample was taken from 3 to 8 mm below the peel. Each sample was packed with silver paper and frozen in liquid nitrogen, and kept at -80 °C until the crude enzyme extraction.

All enzymes extraction procedures were conducted at 4 °C. For phenylalanine ammonia-lyase (PLA), the reaction mixtures contained 1.5 g of frozen sample, 2.5 ml of cold borate buffer (0.1 N, pH 8.8), 1 mmol/l EDTA, 10% (w/v) polyvinylpyrrolidone (PVPP), and 0.05 mM β -mercaptoethanol. For peroxidase (POD) and polyphenoloxidase (PPO), 1.5 g of frozen sample was homogenized in 2.5 ml of cold sodium phosphate buffer (50 mM, pH 7.5), containing 1 mmol/l phenylmethylsulfonyl fluoride (PMSF), 1 mmol/l polyethyleneglycol (PEG), 10% (w/v) PVPP, and 0.1% (v/v) Triton X-100. The mixtures were centrifuged at 12,000g for 30 min and supernatant was used for the enzyme assay.

Supernatant (500 μ l) was incubated with 3 ml L-phenylalanine (7 mM) at 37 °C for 30 min. The enzyme activity was measured at an absorbance of 290 nm using ultraviolet–visible spectrophotometer (PerkinElmer, USA). The PAL activity was expressed as U/mg protein, where one unit was defined as 0.01 Δ OD₂₉₀ per minute.

The reaction mixtures of PPO contained 200 μ l of supernatant, 200 μ l of cold PBS (50 mM, pH 7.0) and 0.5 ml of 50 mM pyrocatechin. The reaction mixtures of POD contained 200 μ l of supernatant, 2.5 ml of 25 mM guaiacol and 200 μ l of 25 mM H₂O₂. The enzyme activity was monitored at 240 nm for 2 min at 24 °C. The PPO and POD activity was expressed as U/mg protein, where 1 U was defined as 0.01 Δ OD₂₉₀ per minute.

Effect of CFS on the contents of total phenolics and falconoids

The froze sample (1.5 g) was homogenized in 2.5 ml of 1% HCl methanol solvent, then centrifuged at 12,000g for 10 min at 4 °C and the supernatant was used for the assay. The total phenol and falconoid content were expressed as Δ OD₂₈₀/g FW and Δ OD₃₂₅/g FW, respectively.

Statistical analysis

All experiments were performed as three independent replicates and expressed as mean $\pm$ standard deviation. Statistical analyses were performed using the Origin Pro 8.0 (Origin Lab Corporation, USA) and SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Antifungal activity of LAB

The inhibitory rate of strain C10, RH-11, LH-9 and DL11 reached 93.93, 89.69, 83.93 and 78.29%; respectively (Fig. 1). The maximum inhibitory rate was exhibited by the CFS of *Lb. plantarum* C10. Therefore, *Lb. plantarum* C10 was selected for further analysis.

Effect of CFS on the spore and mycelial morphology of *T. roseum*

SEM imaging showed that the spores of *T. roseum* appeared to be intact and grew normally in control group (Fig. 2a). When treated with CFS, the seriously damaged and deformed structures of spores were observed, accompanied with intracellular components leakage (Fig. 2b). The mycelial surface of *T. roseum* in control group was smooth with no breakage (Fig. 2c). By contrast, the

Fig. 1 The inhibitory rate of CFS produced by four LAB strains against *T. roseum*. C10: *Lb. plantarum* C10, RH-11: *Lb. rhamnosus* RH-11, LH-9: *Lb. helveticus* LH-9, DL11: *Lb. sakei* DL11

Fig. 2 SEM micrographs of mycelia and spores of *T. roseum* treated with the CFS of *Lb. plantarum* C10. **a** the spores without treated with the CFS of *Lb. plantarum* C10; **b** the spores treated with the CFS of

Lb. plantarum C10; **c** the mycelia without treated with the CFS of *Lb. plantarum* C10; **d** the mycelia treated with the CFS of *Lb. plantarum* C10

mycelial surface of *T. roseum* treated with CFS was collapsed and appeared abnormally club-shaped inflated ends (Fig. 2d). SEM observations indicated that CFS seriously damaged the structures of spores and mycelia, accompanied with cytoplasmic leakage.

Characterization of the antifungal activity of acids mixtures

To determine if the antifungal activity was due to organic acid production, quantification of organic acid in the CFS of *Lb. plantarum* C10 was performed by gas chromatograph analysis. Lactic acid was produced in the highest quantity (61.71 $\mu\text{l/ml}$) and acetic acid (5.83 $\mu\text{l/ml}$) and PLA (5.62 $\mu\text{l/ml}$) were produced in small quantities.

Propionic acid (0.09 $\mu\text{l/ml}$) was produced in trivial amounts.

The inhibitory rate of lactic acid, acetic acid, propionic acid and PLA reached 90.12, 37.54, 3.64 and 9.08%, respectively. Acids mixtures were reconstituted in accordance with the content of each acid existed in the CFS, which showed strongly antifungal activity and inhibitory rate reached 98.18%. The acids mixtures had higher activity than the CFS (93.93%). This result indicated that the acid compounds produced by *Lb. plantarum* C10 were the major antifungal substances.

Effect of pH on antifungal activity of CFS

The maximum antifungal activity was observed at pH 3.5. However, the activity was completely disappeared at pH 7.0 (Fig. 3). This result further indicated that the acid compounds produced by *Lb. plantarum* C10 were the major antifungal substances.

Effect of LAB treatment on fruit decay

The lesion area of fruits inoculated with *T. roseum* and CFS during storage for 7 days is presented in Fig. 4. By day 7, browning and rotting of fruit was observed on positive and negative control groups, and the lesion area of fruit achieved 6259.97 and 6288.05 mm², respectively. By contrast, the evidently reduced account of *T. roseum* contamination was observed on the treated group, and the growth zone of *T. roseum* decreased 19.58 and 13.73%, respectively. This result indicated that CFS significantly reduced contamination zone of *T. roseum* in muskmelons.

Effect of CFS on induction of defense-related enzyme activities

Changes in PAL, POD and PPO activities in response to the CFS are shown in Fig. 5. The activity of PAL was continuously induced by the CFS treatment and reached a peak 36.93 U/mg protein on the sixth days, then declined until the end of storage (Fig. 5a). PAL activity in fruit treated with CFS was 12.0 and 11.6% higher than that in the negative and positive control groups (Fig. 5a). This result suggested that PAL activity in muskmelon was induced by the CFS treatment.

Fig. 3 Effect of pH on the antifungal activity of CFS produced by *Lb. plantarum* C10

The same trend was observed in POD activity. The activity was markedly elevated from 0 to 6 days and then slightly decreased from 6 to 7 days (Fig. 5b). The activity of POD in fruit treated with CFS achieved 2.43 U/mg protein on the sixth days, which was 18.5 and 15.6% higher than that in the negative and positive control groups (Fig. 5b). This result suggested that POD activity in muskmelon was induced by the CFS treatment.

Changes in PPO activity of all groups showed the same trend in which the activity increased initially and then decreased (Fig. 5c). The PPO activity in CFS-treated fruit reached a peak 3.02 U/mg protein on the sixth days, which was 24.3 and 19.8% higher than that in the negative and positive control groups (Fig. 5c). The result indicated that inoculated with CFS markedly induced PPO activity in muskmelon.

Effect of CFS treatment on the contents of total phenolics and flavonoids

Total phenolics content increased to a maximum on the third days and then declined steadily until the end of storage (Fig. 5d). Accumulation of phenolics compounds in fruit by abiotic or biotic elicitors has been considered as a defence mechanism (Wang et al. 2013b). The content of total phenolics treated with CFS reached a peak on the third days, which was 6.3 and 5.0% higher than that in the negative and positive control groups (Fig. 5d). This result indicated that CFS treatment strongly induced accumulation of the total phenolics.

The content of flavonoids declined at 1d and progressively increased to a peak on third days, and then decreased steadily until the end of storage (Fig. 5e). The decrease of flavonoids content at the beginning might be due to the fact of difference in treatment (Sivankalyani et al. 2016). The flavonoids content treated with CFS reached a peak on third days, which was 11.8% higher than that in control groups (Fig. 5e). Accumulation of flavonoids in fruit by biotic elicitors has been regarded as plant defense reactions. This result indicated that the CFS treatment evidently triggered the accumulation of flavonoids in fruit.

Discussion

T. roseum is responsible for major postharvest losses of muskmelon and accounts for up to 10% of the total fungal decay during fruit storage (Yi et al. 2008). The use of LAB bio-preservatives to control plant diseases has been explored as an alternative to the use of synthetic fungicides (Pawlowska et al. 2012; Tripathi and Dubey 2004). Multiple studies have highlighted the antifungal properties of LAB in order to reduce fungal spoilage in fruits (Crowley

Fig. 4 The growth zone of *T. roseum* in muskmelon during storage for 7 days. C10: muskmelon fruit treated with CFS produced by *Lb. plantarum* C10; NC untreated muskmelon fruit used as negative control, PC muskmelon fruit treated with MRS used as positive control

et al. 2013a). Wang et al. (2013a) demonstrated that the shuffled mutant strain *Lb. plantarum* F3C2 inhibit the growth of *Penicillium digitatum* KM08 on the surface of kumquats. In addition, *Lb. plantarum* IMAU10014 was found to have a high ability to prevent *Botrytis cinerea* growth on tomato leaves, and its activity substance include proteinaceous substance and other metabolites (Wang et al. 2011). In this study, the CFS from *Lb. plantarum* C10 exhibited strong antifungal activity towards *T. roseum* and its inhibitory rate reached 93.93%.

It is well known that the role of organic acids as antifungal substances has been widely reported in the literature (Lavermicocca et al. 2000; Prema et al. 2008). Acetic acid and propionic acid are commonly used by manufactures as preservatives in a variety of foods (Gerez et al. 2013). In this study, the contents of lactic acid, acetic acid, propionic acid and PLA in the CFS of *Lb. plantarum* C10 were detected using gas chromatograph analysis. The inhibitory rate of acids mixtures reached 98.18%, which was higher than the CFS. In addition, the antifungal activity of CFS was lost after neutralization treatment, which confirmed the acidic nature of the antifungal substances. These results demonstrated that the organic acids produced by *Lb. plantarum* C10 were the main antifungal substances. Various organic acids produced by LAB have been implemented as fungal inhibitors, where synergistic effects are believed to be involved. For example, a mixture of lactic acid, acetic acid and PLA was identified as being responsible for antifungal activity of *Lb. plantarum* CRL 759, and its antifungal activity was lost after neutralization the pH of the CFS (Gerez et al. 2013).

The antimicrobial effects of organic acids were attributed to reduction of pH to a level below the range of fungi growth (Crowley et al. 2013b). In addition to the effect on pH, undissociated acid could cause the collapse of electrochemical proton gradients, resulting in bacteriostasis and death of susceptible bacteria (Li et al. 2014). The antifungal mechanism of organic acids is quite complicated

and still not fully understood (Torres et al. 2011). In this study, we preliminarily studied the effects of the CFS on the mycelia and spores morphology of *T. roseum* by SEM analysis. The result indicated that the CFS seriously damaged the structures of spores and mycelia, resulted in cytoplasmic leakage. Similar result have been reported for *Lb. casei* AST18, the mycelia and spores of *Penicillium chrysogenum* treated with CFS of *Lb. casei* AST18 were significantly damaged and appeared cytoplasmic leakage (Li et al. 2014). To obtain more detailed information about the antifungal mechanism of LAB, more assays needed to be performed in the future, such as ultrastructure changes, DNA damage and generation of reactive oxygen species.

In recent years, the induction of defense responses in postharvest fruits by various biocontrol agents has become an increasingly attractive option for preventing pathogens (Lu et al. 2013; Wang et al. 2013c; Zhang et al. 2016). Fruits are able to protect themselves upon various phytopathogen attacks by producing a wide spectrum of defence enzymes that improve both cellular protection and disease resistance (Konappa et al. 2016). Defense-related enzymes, including PAL, POD and PPO, are commonly considered important in induced resistance of fruits (Pas-sardi et al. 2004). PAL is a rate-limiting enzyme in the phenylpropanoid pathway, and an increase in PAL activity is associated with biosynthesis of active metabolites such as salicylic acid, phytoalexins, lignins, flavonoids and phenolics (Zhang et al. 2011). Induction of PAL activity increased significantly in fruit surface-wounds following treatment with the CFS. This result is in agreement with previous finding that PAL activity in tomato was enhanced by treatment with *Lb. paracasei* (Konappa et al. 2016).

POD, a biochemical indicator of disease resistance, is a prerequisite for ligin synthesis to reinforce the cell wall and may also alter the antioxidant ability of fruits to cope with pathogens (Brisson et al. 1995). An increase in POD could enhance resistance against pathogen spreading and progress in intercellular spaces of parenchyma cells by

Fig. 5 Effects of the CFS produced by *Lb. plantarum* C10 on the activity of PAL (a), POD (b), PPO (c) and the contents of total phenolics (d) and flavonoids (e) in muskmelon fruit. C10: muskmelon

fruit treated with CFS produced by *Lb. plantarum* C10; NC untreated muskmelon fruit used as negative control, PC muskmelon fruit treated with MRS used as positive control

accelerating the reinforcement of cell walls. Besides POD, rapidly elevated level of PPO is also important for resistant pathogens. PPO can produce antimicrobial quinones through oxidizing polyphenolic compounds (Mohammadi and Kazemi 2002). Compared with the control group, the activities of POD and PPO in CFS-treated muskmelon fruit were obviously enhanced in this study. Similarly, Lu et al. (2013) reported that *Rhodosporidium paludigenum* reduces

the disease incidence caused by *Penicillium digitatum* in citrus fruit and enhances activities of defensive enzymes (PAL, PPO and POD).

Accumulation of phenolics compounds at infection sites may limit the development of the pathogens and lead to rapid cell death. Flavonoids are produced in the phenylpropanoid pathway, which are directly involved in fruit protection against pathogens defense responses. The

concurrent increases in phenolics, flavonoids and activities of defense-related enzymes are consistent with the findings of Konappa et al. (2016), in which *Lb. paracasei* enhanced activities of POD, PPO and PAL and promoted the accumulation of phenolics.

Lb. plantarum C10 treatment significantly reduced the decay incidence and lesion area of muskmelon fruit infected with *T. roseum*. Similar result has been recorded for *Meyerozyma guilliermondii*, treatment with *M. guilliermondii* obviously reduced the decay lesion diameter of pears caused by *P. expansum* (Yan et al. 2018). These results suggested that the CFS produced by *Lb. plantarum* C10, as a biopreservative, effectively reduced pink rot incidence by enhancing the activities of defense-related enzymes.

Conclusion

CFS produced by *Lb. plantarum* C10 strongly inhibited the growth of *T. roseum* and inhibitory rate reached 93.93%. Acid compounds produced by *Lb. plantarum* C10 were the major antifungal substances and exhibited a narrow pH range from 3.5 to 6.5. The antifungal mode of the CFS was to damage the structures of spores and mycelia. When CFS from *Lb. plantarum* C10 was applied to muskmelon, the growth zone of *T. roseum* decreased 19.58% compared to the control, the increased activities of PAL, POD and PPO, and the accumulation of total phenolics and flavanoids were observed. Therefore, *Lb. plantarum* C10 can be used as a biocontrol agent to control the *T. roseum* caused muskmelon fruit rot.

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References

- Brisson LF, Tenhaken R, Lamb C (1995) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* 6:1703–1712. <https://doi.org/10.1105/tpc.6.12.1703>
- Cheong EYL et al (2014) Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mould *Penicillium commune* and their potential as biopreservatives in cheese. *Food Control* 46:91–97. <https://doi.org/10.1016/j.foodcont.2014.05.011>
- Crowley S, Mahony J, van Sinderen D (2013a) Broad-spectrum antifungal-producing lactic acid bacteria and their application in fruit models. *Folia Microbiol* 58:291–299. <https://doi.org/10.1007/s12223-012-0209-3>
- Crowley S, Mahony J, van Sinderen D (2013b) Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. *Trends Food Sci Technol* 33:93–109. <https://doi.org/10.1016/j.tifs.2013.07.004>
- Deng J et al (2015) Postharvest oxalic acid treatment induces resistance against pink rot by priming in muskmelon (*Cucumis melo* L.) fruit. *Postharvest Biol Technol* 106:53–61. <https://doi.org/10.1016/j.postharvbio.2015.04.005>
- Frankova A et al (2016) The antifungal activity of essential oils in combination with warm air flow against postharvest phytopathogenic fungi in apples. *Food Control* 68:62–68. <https://doi.org/10.1016/j.foodcont.2016.03.024>
- Galvez A, Abriouel H, Benomar N, Lucas R (2010) Microbial antagonists to food-borne pathogens and biocontrol. *Curr Opin Biotechnol* 21:142–148. <https://doi.org/10.1016/j.copbio.2010.01.005>
- Ge Y, Deng H, Bi Y, Li C, Liu Y, Dong B (2015) Postharvest ASM dipping and DPI pre-treatment regulated reactive oxygen species metabolism in muskmelon (*Cucumis melo* L.) fruit. *Postharvest Biol Technol* 99:160–167. <https://doi.org/10.1016/j.postharvbio.2014.09.001>
- Gerez CL, Torres MJ, Font de Valdez G, Rollán G (2013) Control of spoilage fungi by lactic acid bacteria. *Biol Control* 64:231–237. <https://doi.org/10.1016/j.biocontrol.2012.10.009>
- Ghosh R, Barman S, Mukhopadhyay A, Mandal NC (2015) Biological control of fruit-rot of jackfruit by rhizobacteria and food grade lactic acid bacteria. *Biol Control* 83:29–36. <https://doi.org/10.1016/j.biocontrol.2014.12.020>
- Konappa NM, Maria M, Uzma F, Krishnamurthy S, Nayaka SC, Niranjana SR, Chowdappa S (2016) Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against *Ralstonia solanacearum* causing bacterial wilt. *Sci Hortic* 207:183–192. <https://doi.org/10.1016/j.scienta.2016.05.029>
- Lan WT, Chen YS, Wu HC, Yanagida F (2012) Bio-protective potential of lactic acid bacteria isolated from fermented wax gourd. *Folia Microbiol* 57:99–105. <https://doi.org/10.1007/s12223-012-0101-1>
- Lavermicocca P, Valerio F, Evidente A, Lazzaroni S, Corsetti A, Gobbetti M (2000) Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Appl Environ Microbiol* 66:4084–4090. <https://doi.org/10.1128/aem.66.9.4084-4090.2000>
- Li H et al (2014) Antifungal activities and effect of *Lactobacillus casei* AST18 on the mycelia morphology and ultrastructure of *Penicillium chrysogenum*. *Food Control* 43:57–64. <https://doi.org/10.1016/j.foodcont.2014.02.045>
- Lu L et al (2013) *Rhodosporidium paludigenum* induces resistance and defense-related responses against *Penicillium digitatum* in citrus fruit. *Postharvest Biol Technol* 85:196–202. <https://doi.org/10.1016/j.postharvbio.2013.06.014>
- Mohammadi M, Kazemi H (2002) Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. *Plant Sci* 162:491–498. [https://doi.org/10.1016/S0168-9452\(01\)00538-6](https://doi.org/10.1016/S0168-9452(01)00538-6)
- Passardi F, Penel C, Dunand C (2004) Performing the paradoxical: how plant peroxidases modify the cell wall. *Trends Plant Sci* 9:534–540. <https://doi.org/10.1016/j.tplants.2004.09.002>
- Pawlowska AM, Zannini E, Coffey A, Arendt EK (2012) “Green preservatives”: combating fungi in the food and feed industry by applying antifungal lactic acid bacteria. *Adv Food Nutr Res* 66:217–238. <https://doi.org/10.1016/B978-0-12-394597-6.00005-7>
- Plaza L, Altisent R, Alegre I, Viñas I, Abadias M (2016) Changes in the quality and antioxidant properties of fresh-cut melon treated with the biopreservative culture *Pseudomonas graminis* CPA-7

- during refrigerated storage. *Postharvest Biol Technol* 111:25–30. <https://doi.org/10.1016/j.postharvbio.2015.07.023>
- Prema P, Smila D, Palavesam A, Immanuel G (2008) Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. *Food Bioprocess Technol* 3:379–386. <https://doi.org/10.1007/s11947-008-0127-1>
- Sivankalyani V, Feygenberg O, Diskin S, Wright B, Alkan N (2016) Increased anthocyanin and flavonoids in mango fruit peel are associated with cold and pathogen resistance. *Postharvest Biol Technol* 111:132–139. <https://doi.org/10.1016/j.postharvbio.2015.08.001>
- Terry L (2004) Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. *Postharvest Biol Technol* 32:1–13. <https://doi.org/10.1016/j.postharvbio.2003.09.016>
- Torres R, Teixidó N, Usall J, Abadías M, Mir N, Larrigaudiere C, Viñas I (2011) Anti-oxidant activity of oranges after infection with the pathogen *Penicillium digitatum* or treatment with the biocontrol agent *Pantoea agglomerans* CPA-2. *Biol Control* 57:103–109. <https://doi.org/10.1016/j.biocontrol.2011.01.006>
- Tripathi P, Dubey NK (2004) Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biol Technol* 32:235–245. <https://doi.org/10.1016/j.postharvbio.2003.11.005>
- Tsuda K et al (2016) Biological control of bacterial soft rot in Chinese cabbage by *Lactobacillus plantarum* strain BY under field conditions. *Biol Control* 100:63–69. <https://doi.org/10.1016/j.biocontrol.2016.05.010>
- Wang H, Yan H, Shin J, Huang L, Zhang H, Qi W (2011) Activity against plant pathogenic fungi of *Lactobacillus plantarum* IMAU10014 isolated from Xinjiang koumiss in China. *Ann Microbiol* 61:879–885. <https://doi.org/10.1007/s13213-011-0209-6>
- Wang H et al (2013a) Genome shuffling of *Lactobacillus plantarum* for improving antifungal activity. *Food Control* 32:341–347. <https://doi.org/10.1016/j.foodcont.2012.12.020>
- Wang J, Bi Y, Wang Y, Deng J, Zhang H, Zhang Z (2013b) Multiple preharvest treatments with harpin reduce postharvest disease and maintain quality in muskmelon fruit (cv. Huanghemi). *Phytoparasitica* 42:155–163. <https://doi.org/10.1007/s12600-013-0351-8>
- Wang X, Xu F, Wang J, Jin P, Zheng Y (2013c) *Bacillus cereus* AR156 induces resistance against Rhizopus rot through priming of defense responses in peach fruit. *Food Chem* 136:400–406. <https://doi.org/10.1016/j.foodchem.2012.09.032>
- Yan Y et al (2018) Control of postharvest blue mold decay in pears by *Meyerozyma guilliermondii* and its effects on the protein expression profile of pears. *Postharvest Biol Technol* 136:124–131. <https://doi.org/10.1016/j.postharvbio.2017.10.016>
- Yang EJ, Chang HC (2010) Purification of a new antifungal compound produced by *Lactobacillus plantarum* AF1 isolated from kimchi. *Int J Food Microbiol* 139:56–63. <https://doi.org/10.1016/j.ijfoodmicro.2010.02.012>
- Yang B, Yongcai L, Yonghong G, Yi W (2009) Induced resistance in melons by elicitors for the control of postharvest diseases. *Postharvest Pathol* 2:31–41. https://doi.org/10.1007/978-1-4020-8930-5_3
- Yi W, Xuan LI, Yang BI, Yong-Hong GE, Yong-Cai LI, Fang X (2008) Postharvest ASM or harpin treatment induce resistance of muskmelons against *Trichothecium roseum*. *Agric Sci China* 7:217–223. [https://doi.org/10.1016/S1671-2927\(08\)60042-5](https://doi.org/10.1016/S1671-2927(08)60042-5)
- Zhang Z, Bi Y, Ge Y, Wang J, Deng J, Xie D, Wang Y (2011) Multiple pre-harvest treatments with acibenzolar-S-methyl reduce latent infection and induce resistance in muskmelon fruit. *Sci Hortic* 130:126–132. <https://doi.org/10.1016/j.scienta.2011.06.024>
- Zhang Q et al (2016) *Streptomyces rochei* A-1 induces resistance and defense-related responses against *Botryosphaeria dothidea* in apple fruit during storage. *Postharvest Biol Technol* 115:30–37. <https://doi.org/10.1016/j.postharvbio.2015.12.013>