https://doi.org/10.17221/175/2020-CJFS

Screening of *Lactobacillus plantarum* with broad-spectrum antifungal activity and its application in preservation of golden-red apples

*Kangning Li, Wenyi Zhang, Lai-Yu Kwok, Bilige Menghe**

Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Key Laboratory of Dairy Products Processing, Ministry of Agriculture and Rural Affairs, Inner Mongolia Key Laboratory of Dairy Biotechnology and Engineering, Inner Mongolia Agricultural University, Huhhot, P.R. China

**Corresponding author: mhblg@163.com*

Citation: Li K., Zhang W., Kwok L.Y., Menghe B. (2020): Screening of *Lactobacillus plantarum* with broad-spectrum antifungal activity and its application in preservation of golden-red apples. Czech J. of Food Sci., 38: 315–322.

Abstract: Fungal food spoilage is a common problem that leads to both great economic losses and serious health problems. This study screened the antifungal activity of 137 *Lactobacillus plantarum* isolates against six common food spoilage indicator fungi using an overlay method and indicator strains of the species *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium expansum, Penicillium chrysogenum, Cladosporium cladosporioides*, and *Aspergillus niger*. Among *Lactobacillus plantarum* isolates, strain IMAU80174 was selected as the most effective based on the results of mycelium growth inhibition by its cell-free supernatant (CFS) and tolerance to simulated gastrointestinal juices and bile. The CFS of *Lactobacillus plantarum* IMAU80174 showed heat and protease resistance, and it was active only in a low pH environment. The application of the CFS to golden-red apples could slow down spoilage caused by inoculation of *Penicillium expansum*.

Keywords: Lactic acid bacteria, antifungal properties, antifungal compounds, biopreservation, fruit

Fungal food spoilage is a main cause that leads to great economic losses and serious health-related problems (Pitt & Hocking 2009). The most common food spoilage fungal species belong to the genera *Aspergillus*, *Penicillium* (Le Lay et al. 2016a), *Cladosporium*, and *Fusarium* (Dalié et al. 2010). In order to inhibit the activity of food spoilage fungi so as to extend product shelf life, different methods including heat treatment, modified atmosphere packaging, and addition of chemical preservatives have been developed (Schnürer et al. 2005). However, some of these methods could deteriorate the sensory quality of the products or might involve the addition of food preservatives that might have potential health effects. Thus, to minimize the potential undesirable effects, alternative methods such as the supplementation of natural preservatives have been investigated (Axel et al. 2017).

L. plantarum is a versatile and widespread species found in different environments, ranging from food to animal and human gastrointestinal tracts (De Vries et al. 2006; Papadimitriou et al. 2015). This species is an ideal candidate for use in biopreservation against food spoilage fungi due to its generally regarded as safe (GRAS) status. Especially, a previous study showed that *L. plantarum* has a stronger and broader spectrum of fungal inhibitory activity than other *Lactobacillus* species (Lavermicocca et al. 2000; Sevgi & Ignatova-Ivanova 2015). Further studies have confirmed that the antifungal activity of *L. plantarum* was related not only to competitive growth but also

Supported by the National Natural Science Foundation of China, Grant No. 31660450.

https://doi.org/10.17221/175/2020-CJFS

to a wide variety of active antifungal compounds such as organic acid, phenyllactic acid, 3-phenyllactic acid, cyclic dipeptide (Niku-Paavola et al. 1999; Lavermicocca et al. 2000; Ström et al. 2002; Bianchini & Bullerman 2009; Prema et al. 2010; Sangmanee & Hongpattarakere 2014). A published study has successfully applied *L. plantarum* FST1.7 strain in sourdough to increase the shelf life of wheat bread by inhibiting the growth of *Fusarium* (Dal Bello et al. 2007). Pretreatment of food and feed with cell-free supernatants (CFS) of *L. plantarum* MYS44 could reduce or eliminate aflatoxin B_1 production by inhibiting the toxin-producing fungus *Aspergillus parasiticus* (Rao et al. 2019). At the same time, commercial solutions are gradually being developed and applied, for example, Holdbac® YM-XPM and YM-XPK (DuPont Danisco, China) containing *L. plantarum* are used to inhibit the growth of fungi in dairy products. All these works have demonstrated the suitability of using *L. plantarum* as a natural biopreservative in modern food industry.

The aim of this study was to screen the antifungal activity of 137 *L. plantarum* isolates against six common food-spoilage fungi and to preliminarily characterise several isolates that showed a strong and broad spectrum of antifungal activity. Finally, the spoilage protection effect of a selected strain was tested in golden-red apple. This work helped identify strains that were suitable for use as natural biopreservatives in food.

MATERIAL AND METHODS

Bacterial strains and cultivation conditions. One hundred and thirty-seven isolates of *L. plantarum* available at the Lactic Acid Bacteria Collection Center (LABCC) of the Key Laboratory of Dairy Biotechnology and Engineering, Inner Mongolia Agricultural University, Inner Mongolia, People's Republic of China, were used in this work. These strains were previously isolated from different food matrices and identified based on 16S ribosomal RNA gene sequence analysis. All isolates were stored at –80 °C in 30% skimmed milk medium and were routinely grown in MRS broth (Oxoid Ltd., England) at 37 °C for 24 h in the current experiments.

Fungal strains and cultivation conditions. Six common food spoilage fungi were chosen as indicator fungi in this work. Three of them, *Aspergillus (A.)* flavus CICC 2219, *Fusarium (F.) moniliforme* CICC 2490, and *Cladosporium (C.) cladosporioides* CICC 2477, were obtained from the China Center of Industrial Culture Collection (CICC, Beijing, China), while the other three, *Penicillium (P.) chrysogenum* BNCC 185782, *P. expansum* BNCC 185786, and *A. niger* BNCC 186328, were purchased from the BeNa Culture Collection (BNCC, Beijing, China). The lyophilized fungal cultures were resuspended in sterile distilled water and recovered in potato dextrose agar (PDA, Oxoid Ltd., England) slants by culturing at 25 °C for 5–7 days or until sporulation. The fungal spore suspensions were filtered with five sterile layers of gauze to separate the spores from mycelia. The spore density was estimated by the spread plate method, and the concentration of spores was adjusted to around 10^6 spores mL^{-1}.

Antifungal activity assays of *L. plantarum* isolates. An aliquot of 5 µL of *L. plantarum* culture was spotted on an MRS agar plate for the assays. After 24 h of incubation at 37 °C, the plates were overlaid with 10 mL of PDA containing 0.1 mL (106 spores mL^{-1}) of each fungus and were incubated aerobically at 25 °C for 5 days (Rouse et al. 2008). Antifungal activities of the *L. plantarum* strains were assessed based on the inhibition zone size around the bacterial spot: no inhibition (–), no clear zone; weak inhibition (+), an inhibition zone of 1–6 mm; moderate inhibition (++), an inhibition zone of 6–12 mm; strong inhibition (+++), an inhibition zone of more than 12 mm. The inhibition zone size was calculated as the diameter of fungal inhibition minus the diameter of *L. plantarum* growth.

Mycelium growth inhibition by *L. plantarum* CFS. *L. plantarum* strains were grown in MRS broth at 37 °C for 24 h. The corresponding CFS were obtained by centrifugation at 8 000 × g for 5 min at 4 °C and filtered by a sterile 0.22 µm membrane. A 2 mL aliquot of CFS was mixed with 18 mL of PDA (10%, v/v), and the mixture was poured into sterile Petri dishes. After the agar solidified, the indicator fungus (3–5 mm diameter culture of growing fungal mycelia) was inoculated in the middle of the Petri dishes. The plate was allowed to incubate at 25 °C for 7 days. Control plates containing 18 mL of PDA mixed with 2 mL of MRS broth were processed in parallel (Le Lay et al. 2016a). The antifungal activity was calculated as follows:

$$I = \frac{C - T}{C} \times 100\,(\%) \quad (1)$$

where: I – the hyphal radial growth inhibition rate; C – the diameter of mycelial growth on control plates (mm); T – the diameter of mycelial growth on test plates containing CFS (mm).

Preliminary characterisation of antifungal compounds of CFS. The CFS was heat-treated for 30 min at 100 °C (CFS-A) or neutralised with 2 M NaOH (adjusted to pH 6.5) (CFS-B). Then the neutralized supernatant

https://doi.org/10.17221/175/2020-CJFS

(CFS-B) was incubated with proteinase K (1 mg mL^{-1}; Sigma-Aldrich, USA) for 1 h, and the pH was readjusted to the initial value with 2 M HCl (CFS-C). The treatment effect was measured by the mycelium inhibition assay as described above (Russo et al. 2017).

Quantification of organic acids and phenolic acid in CFS. Organic acids (lactic, acetic, propionic) and phenolic acid (phenyllactic and 4-hydroxyphenyllactic acid) were determined in the CFS of 24 h by Sciex Qtrap 6500+ LC-MS/MS system (AB Sciex, USA). Sample preparation and quantitative analysis method were done as described by Han et al. (2015).

Tolerance of selected *L. plantarum* to artificial gastrointestinal juices. *L. plantarum* strains were grown in MRS broth at 37 °C for 12 h. Cells were collected by centrifugation, washed twice with PBS, and resuspended in the same buffer. The simulated gastric juice was a preparation of 0.3% (w/v) pepsin (Sigma-Aldrich, USA) adjusted to pH 2.5, followed by filtered sterilisation. The simulated intestinal juice was a mixture of 0.1% (w/v) trypsin (Sigma-Aldrich, USA) and 0.3% (w/v) bile (Sigma-Aldrich, USA) adjusted to pH 8.0. An aliquot of 0.5 mL of *L. plantarum* cell suspension was mixed with 4.5 mL of the simulated gastric juice, and the mixture was incubated at 37 °C for 3 h. Then 0.5 mL of the cultured mixture was further inoculated into 4.5 mL of the simulated intestinal juice at 37 °C, followed by anaerobic incubation for 8 h (Li et al. 2017). An aliquot of 0.5 mL of the bacteria-gastric juice mixture was sampled at 0 h and 3 h after incubation, as well as after 4 h and 8 h of incubation in simulated intestinal juice plated. The bacteria-simulated digestive juice mixtures were plated on MRS agar with appropriate dilutions and incubated anaerobically for 48 h at 37 °C to calculate the total viable counts. Data were expressed as $\log_{10}$ CFU mL^{-1}.

The survival rate was calculated according to the following equation:

$$\text{Survival rate} = \frac{\log N_1}{\log N_0} \times 100\,(\%) \quad (2)$$

where: N_0 and N_1 are the total viable counts of *L. plantarum* before and after the treatment, respectively.

Tolerance of selected *L. plantarum* to bile. The selected strains (1%, v/v) were mixed with MRS broth containing 0.2% w/v sodium thioglycolate (Kanto, Japan) with and without 0.3% (w/v) oxgall (DIFCO, Canada), respectively. The mixture was incubated at 37 °C for 12 h, and changes in absorbance at 620 nm were monitored. The delay time of oxgall-containing culture in reaching an absorbance value of 0.3 in comparison with the culture without oxgall was considered as the lag time (Walker & Gilliland 1993).

Protection of golden-red apples from fungal spoilage by CFS. Golden-red apples commercially available, mature, of uniform size, pH range from 4.19 to 4.58, without spoilage were selected for the assay. The apples were washed with sterile distilled water and dried for 30 min. Each fruit was punched a 5 mm diameter wound with a 1 mL sterile pipette tip. Then 5 µL of *Penicillium expansum* conidia suspension (10^6 spores mL^{-1}) and 50 µL of *L. plantarum* CFS were inoculated together in the wound. The inoculated apples were incubated for 7 days at 25 °C. The negative control (without fungus) and the positive control (inoculated only with the fungus but not with CFS) were processed in parallel.

Statistical Analysis. Three independent replicates of each experiment were obtained. All data were presented as mean ± standard deviation (SD). Significant differences between groups were evaluated by analysis of variance (ANOVA) using the software package SPSS 22. A value $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Antifungal activity assays of *L. plantarum* strains. To identify *L. plantarum* that possessed wide-spectrum antifungal activity, 137 *L. plantarum* isolates were screened against six indicator fungi using an overlay method (Figure 1). The species *A. niger* was the most resistant fungal isolate because 74% of the tested *L. plantarum* strains were unable to inhibit its growth, while the remaining 26% of the isolates showed only weak antifungal activity. In contrast, 71% and 98% of *L. plantarum* isolates exerted moderate or strong antifungal activity against the strains of *F. moniliforme* and *C. cladosporioides*, respectively. In addition, between 22% and 41% of the tested *L. plantarum* isolates showed moderate or strong antifungal activity against *A. flavus*, *P. expansum*, and *P. chrysogenum*. Finally, in all *L. plantarum* isolates, only a few isolates could inhibit all six indicator fungi, and ten *L. plantarum* strains were selected for further investigation (Table S1).

Our results are consistent with some published works. For example, a recent work screened the antifungal activities of 270 LAB strains and only a low proportion of the screened strains could suppress the growth of *A. niger* (Le Lay et al. 2016b). Another study reported that 88 *L. plantarum* strains exhibited strong inhibitory activity against *P. chrysogenum, P. expansum*, and *F. culmorum* (Russo et al. 2017).

https://doi.org/10.17221/175/2020-CJFS

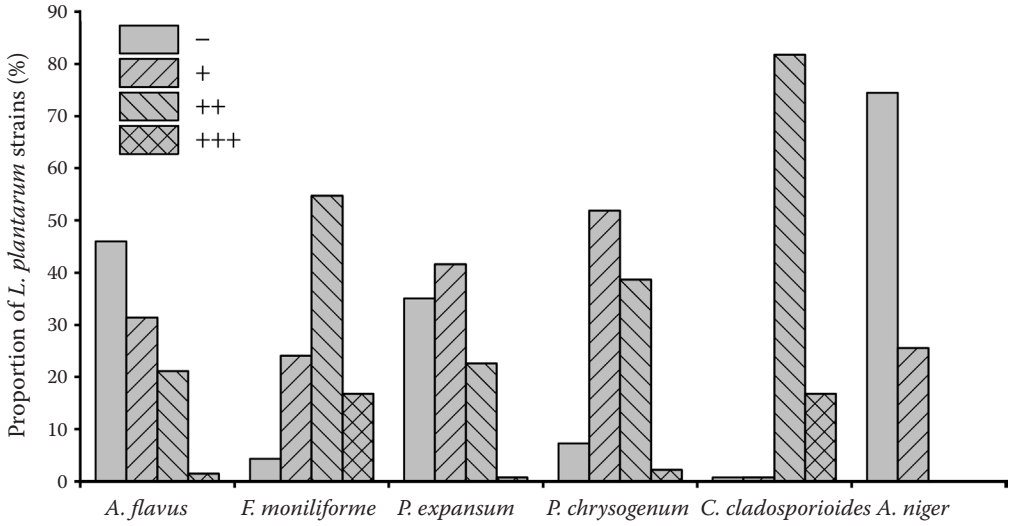

Figure 1. Antifungal activity of 137 *L. plantarum* strains against six indicator fungi by the overlay method
Antifungal activity of *L. plantarum* strains was classified as: (–) no; (+) weak; (++) moderate; (+++) strong

Antifungal activity of CFS of selected *L. plantarum* isolates. The antifungal activity of LAB can be achieved either directly through competition among living cells or indirectly through secretion of active antagonistic metabolites. To verify the antifungal activity of the ten selected *L. plantarum* isolates, the effect of their CFS on mycelial growth inhibition was determined by assay. The results showed that the CFS of all ten strains could not inhibit the growth of *A. niger* BNCC186328. In contrast, all the tested *L. plantarum* CFS exhibited a variable inhibition response towards the other five indicator fungi. Among them, the isolates IMAU10382, IMAU80174, and IMAU10418 showed an overall higher inhibitory fungal activity compared with the other seven strains (Table S2).

A previous report has shown that the addition of CFS (12%, v/v) of *L. plantarum* UFG 108 and UFG 121 to the culture media conferred a strong antifungal activity, with a hyphal radial growth inhibition rate of 50% and 60% against *P. expansum* and *F. culmorum*, respectively (Russo et al. 2017). Another study found that the CFS of *L. plantarum* MYS44 (6%) exhibited the maximum inhibition of growth against *A. parasiticus* (Rao et al. 2019). The antifun gal activity patterns of the CFS of the ten selected *L. plantarum* isolates were largely consistent with those conferred by live cells determined in the preliminary overlay assay, except that the CFS was not effective in inhibiting *A. niger* at all. This could be due to an insufficient concentration of CFS (10%) used in the assay or the possibility that the inhibitory mechanism was at least partially cell-based.

Tolerance of selected *L. plantarum* isolates to simulated gastrointestinal juices and bile. The ability to survive the gastrointestinal tract transit is considered as an important criterion of probiotics. So, the tolerance of selected isolates (IMAU10382, IMAU80174 and IMAU10418) to simulated gastrointestinal juices and bile was evaluated. The isolate IMAU80174 had the highest tolerance and survival rates when

Table 1. Tolerance of three *Lactobacillus plantarum* strains to simulated gastrointestinal juices

Lactobacillus plantarum strains	Survival in simulated gastric juice at pH 2.5			Survival in simulated intestinal juice at pH 8			
	0 h	3 h	3 h	4 h	8 h	4 h	8 h
	($\log_{10}$ CFU mL^{-1})		(%)	($\log_{10}$ CFU mL^{-1})		(%)	
IMAU10382	9.04 ± 0.06	5.87 ± 0.04	64.87 ± 0.39^{b}	5.50 ± 0.05	5.23 ± 0.05	60.85 ± 0.21^{b}	57.88 ± 0.33^{b}
IMAU10418	8.70 ± 0.02	5.72 ± 0.04	65.77 ± 0.49^{b}	5.32 ± 0.06	5.12 ± 0.09	61.17 ± 0.53^{b}	58.90 ± 0.97^{b}
IMAU80174	8.82 ± 0.06	6.91 ± 0.07	78.37 ± 0.46^{a}	6.62 ± 0.07	6.39 ± 0.34	75.14 ± 1.05^{a}	72.50 ± 4.08^{a}

Data are expressed as mean ± SD; experiments were done in triplicate; statistically significant differences between strains within each column are indicated by different letters ($P < 0.05$)

https://doi.org/10.17221/175/2020-CJFS

Table 2. Tolerance of three *Lactobacillus plantarum* strains to 3% oxgall

Lactobacillus plantarum strains	Time needed to increase the absorbance by 0.3 at 620 nm (h)		Lag time (h)
	no oxgall	0.3% (w/v) oxgall	
IMAU10382	5.23 ± 0.55	6.13 ± 0.51	0.91 ± 0.05[a]
IMAU10418	5.12 ± 0.32	6.06 ± 0.37	0.94 ± 0.05[a]
IMAU80174	3.70 ± 0.02	4.58 ± 0.35	0.88 ± 0.35[a]

Data are expressed as mean ± SD; experiments were done in triplicate; statistically significant differences between strains within each column are indicated by different letters ($P < 0.05$)

treated with simulated gastric juice and intestinal juice ($P < 0.05$) (Table 1). No significant difference was found between the three tested strains in the lag time when grown in the presence of bile salt ($P > 0.05$) (Table 2).

A well-studied probiotic strain, *L. plantarum* DSM 2648, showed good tolerance to gastrointestinal conditions, and there was no loss in cell viability after incubating for 4 h at pH 4.0 and 0.5% bile in an *in vitro* assay (Anderson et al. 2010). Similar results were observed for *L. plantarum* LZ95 and CY3; over 90% of cells survived 4- hour incubation in simulated gastric juice and 0.3% bile salt (Li et al. 2017). Our data showed that *L. plantarum* IMAU80174 exhibited moderate tolerance towards simulated digestive juices and 3% oxgall (survival rate of 78.37 ± 0.46%).

Resistance of antifungal activity of *L. plantarum* IMAU80174 CFS towards heat, alkali, and proteinase K treatments. Based on the antifungal profile and tolerance to simulated digestive juices and bile, the CFS of the isolate IMAU80174 was further characterised for its resistance towards heat, alkali, and proteinase K treatments. A repeated assay showed a similar pattern of inhibition against all five indicator fungi. No significant reduction of antifungal activity was observed after heat and proteinase K treatments. However, the antifungal activity was drastically reduced when the pH of CFS was adjusted to a relatively neutral value of pH 6.5 ($P < 0.05$ in all cases) (Figures 2 and 3).

A previous study found that the acidity in the microenvironment might influence the efficacy of antifungal activity (Suhr & Nielsen 2004; Lv et al. *2018*). In addition to acidity, some bioactive substances were sensitive to heat or protease treatment (Le Lay et al. 2016a; Muhialdin et al. 2018). The *L. plantarum* IMAU80174 CFS fungal activity was active only in the acidic environment and its heat- and protease-

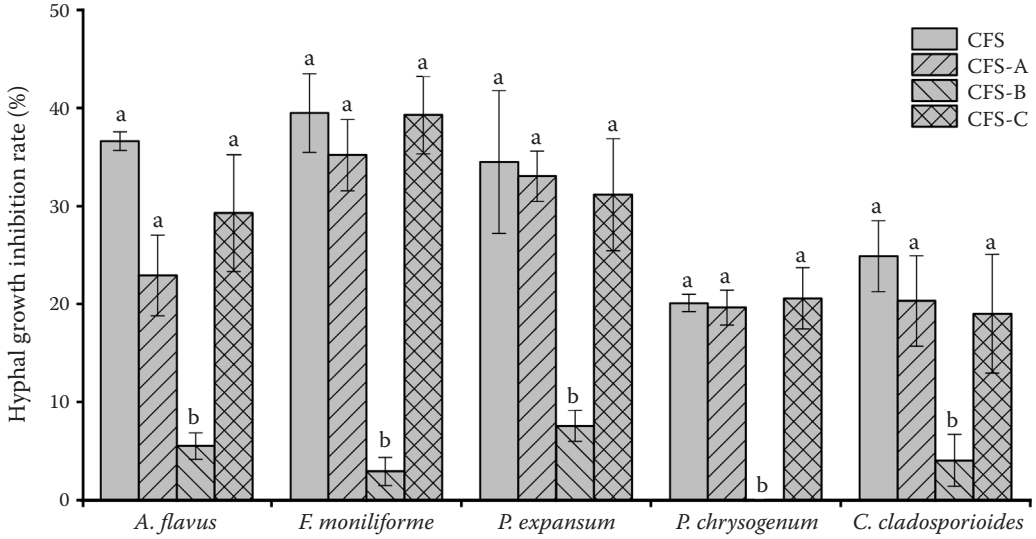

Figure 2. Growth inhibition rate of different treatments with the CFS from *L. plantarum* IMAU80174 against indicator fungi for 7 days at 25 °C

Data points are expressed as mean ± SD of triplicate replications; statistically significant differences are indicated by different letters ($P < 0.05$)

https://doi.org/10.17221/175/2020-CJFS

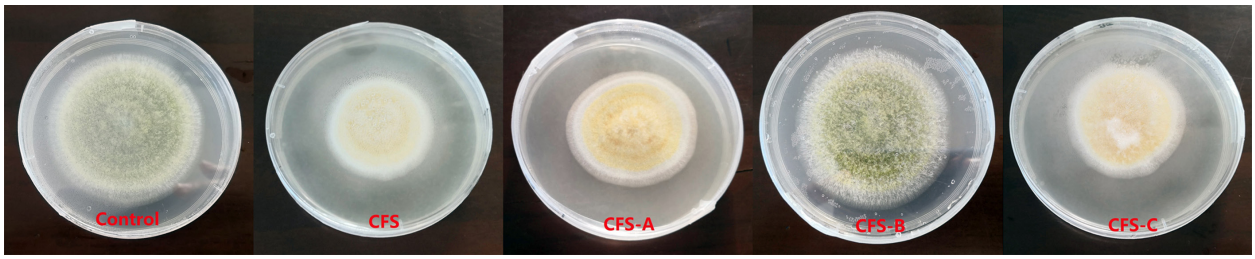

Figure 3. Examples of inhibiting *A. flavus*: Control – no CFS; CFS – untreated CFS; CFS-A – heat treated for 30 min at 100 °C, CFS-B – neutralized with 2M NaOH (6.5); CFS-C – incubated with 1 mg mL^{-1} proteinase K for 1 h

resistant activity might suggest a non-proteinaceous nature of the antifungal materials.

Generally, some research reported that organic acids and phenolic acids had antifungal activities (Russo et al. 2017). Therefore, the CFS of *L. plantarum* IMAU80174 was analysed by LC-MS/MS after 24-hour fermentation. All five acids were detected and the concentrations of lactic acid, acetic acid, propionic acid, phenyllactic acid and 4-hydroxyphenyllactic acid were 3.53 g L^{-1}, 0.88 g L^{-1}, 0.88 mg L^{-1}, 9.17 mg L^{-1} and 6.14 mg L^{-1}, respectively.

Previous studies showed that lactic acid alone had a low antifungal activity, but the synergistic antifungal effect of lactic acid and acetic acid was enhanced (Le Lay et al. 2016a). As expected, phenyllactic and 4-hydroxyphenyllactic acids were found in the CFS of *L. plantarum* IMAU80174, but their concentrations were far from reaching the inhibition of fungal growth. So, lactic and acetic acid may be the major antifungal substances of the CFS of *L. plantarum* IMAU80174, other acids like propionic, phenyllactic and 4-hydroxyphenyllactic acid may have a synergistic effect at a low concentration.

Protection of golden-red apple spoilage. *P. expansum* was chosen as it is a natural pathogen of the apple disease and spoilage by producing patulin and initiating infection often at sites of fruit injury.

P. expansum was inoculated to the golden-red apple alone, with or without CFS of *L. plantarum* IMAU80174. After seven days of incubation, obvious differences were found in the average diameter of the browning and rotting area between the negative control (without *P. expansum*; no rotting area), positive control (inoculated with *P. expansum* without CFS; 39.82 ± 5.30 mm) and the experimental group (inoculated with both *P. expansum* and CFS; 23.91 ± 3.08 mm) (Figure 4).

To protect from food spoilage, the species *L. plantarum* has been successfully applied as a biopreservative in a wide range of food, including cereals-based foods, wild cherries, peanuts, and muskmelon (Lipińska et al. 2016; Russo et al. 2017; Lv et al. 2018; Rao et al. 2019). Our results suggested that the application of *L. plantarum* CFS could effectively decrease the rate of decay and lesion in apples subject to inoculation with the spoilage fungus *P. expansum*. Thus, it is worth fur-

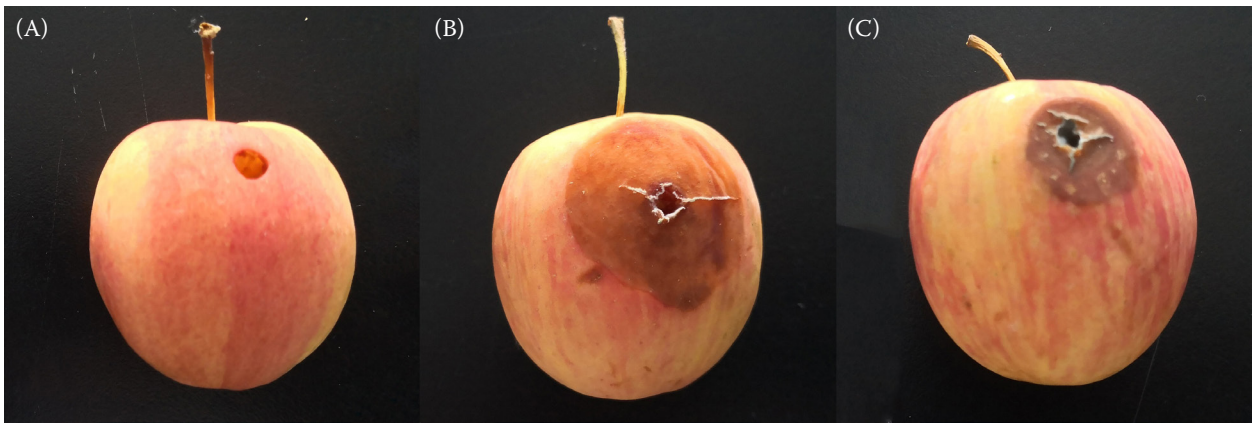

Figure 4. The contamination zone of *P. expansum* in golden-red apple during storage at 25 °C for 7 days: negative control – without *P. expansum* (A), positive control – infected by *P. expansum* (B), *P. expansum* and CFS of *L. plantarum* IMAU80174 were inoculated (C)

https://doi.org/10.17221/175/2020-CJFS

ther exploring the potential of applying *L. plantarum* as a biopreservative for apples and other fruits.

CONCLUSION

The antifungal activity of 137 *L. plantarum* strains was studied using an overlay method against the six common indicator and food spoilage fungi. The antifungal activities of CFS from selected isolates and their resistance to heat, acidity, and protease were characterised. The isolate IMAU80174 was found to have an overall strong and broad spectrum of antifungal activity, and its CFS was highly resistant to heat and protease. However, the efficacy of the antifungal activity was limited to an acidic range. Finally, this isolate could also slow down the decay rate of golden-red apples subject to inoculation by the spoilage fungus *P. expansum*. Taken altogether, this isolate has a good potential to serve as a biopreservative, which needs to be further explored.

REFERENCES

Anderson R.C., Cookson A.L., McNabb W.C., Kelly W.J., Roy N.C. (2010): *Lactobacillus plantarum* DSM 2648 is a potential probiotic that enhances intestinal barrier function. FEMS Microbiology Letters, 309: 184–192.

Axel C., Zannini E., Arendt E.K. (2017): Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf life extension. Critical Reviews in Food Science and Nutrition, 57: 3528–3542.

Bianchini A., Bullerman L.B. (2009). Biological control of molds and mycotoxins in foods. Acs Symposium Series, Oxford University Press, 1031: 1–16.

Dal Bello F., Clarke C.I., Ryan L.A.M., Ulmer H., Schober T.J., Ström K., Sjögren J., Van Sinderen D., Schnürer J., Arendt E.K. (2007): Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. Journal of Cereal Science, 45: 309–318.

Dalié D.K.D., Deschamps A.M., Richard-Forget F. (2010) Lactic acid bacteria – Potential for control of mould growth and mycotoxins: A review. Food Control, 21: 370–380.

De Vries M.C., Vaughan E.E., Kleerebezem M., De Vos W.M. (2006): *Lactobacillus plantarum* – survival, functional and potential probiotic properties in the human intestinal tract. International Dairy Journal, 16: 1018–1028.

Han J., Lin K., Sequeira C., Borchers C.H. (2015): An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography–tandem mass spectrometry. Analytica Chimica Acta, 854: 86–94.

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. Applied and Environmental Microbiology, 66: 4084–4090.

Le Lay C., Coton E., Le Blay G., Chobert J.M., Haertlé T., Choiset Y., Van Long N.N., Meslet-Cladière L., Mounier J. (2016a): Identification and quantification of antifungal compounds produced by lactic acid bacteria and propionibacteria. International Journal of Food Microbiology, 239: 79–85.

Le Lay C., Mounier J., Vasseur V., Weill A., Le Blay G., Barbier G., Coton E. (2016b): *In vitro* and in situ screening of lactic acid bacteria and propionibacteria antifungal activities against bakery product spoilage molds. Food Control, 60: 247–255.

Li P., Gu Q., Yang L., Yu Y., Wang Y. (2017): Characterization of extracellular vitamin B12 producing *Lactobacillus plantarum* strains and assessment of the probiotic potentials. Food Chemistry, 234: 494–501.

Lipińska L., Klewicki R., Klewicka E., Kołodziejczyk K., Sójka M., Nowak, A. (2016): Antifungal activity of *lactobacillus* sp. bacteria in the presence of xylitol and galactosyl-xylitol. Journal of Biomedicine and Biotechnology, 2016: 1–8.

Lv X., Ma H., Lin Y., Bai F., Ge Y., Zhang D., Li J. (2018): Antifungal activity of *Lactobacillus plantarum* C10 against *Trichothecium roseum* and its application in promotion of defense responses in muskmelon (*Cucumis melo* L.) fruit. Journal of Food Science and Technology, 55: 3703–3711.

Muhialdin B.J., Hassan Z., Saari N. (2018): *In vitro* antifungal activity of lactic acid bacteria low molecular peptides against spoilage fungi of bakery products. Annals of Microbiology, 68: 557–567.

Niku-Paavola M.L., Laitila A., Mattila-Sandholm T., Haikara A. (1999): New types of antimicrobial compounds produced by *Lactobacillus plantarum*. Journal of Applied Microbiology, 86: 29–35.

Papadimitriou K., Pot B., Tsakalidou E. (2015): How microbes adapt to a diversity of food niches. Current Opinion in Food Science, 2: 29–35.

Pitt J.I., Hocking A.D. (2009). Fungi and food spoilage. Springer, New York: 401–421.

Prema P., Smila D., Palavesam A., Immanuel G. (2010): Production and characterization of an antifungal compound (3-phenyllactic acid) produced by *Lactobacillus plantarum* strain. Food and Bioprocess Technology, 3: 379–386.

Rao K.P., Deepthi B.V., Rakesh S., Ganesh T., Achar P., Sreenivasa M.Y. (2019): Antiaflatoxigenic Potential of Cell-Free Supernatant from *Lactobacillus plantarum* MYS44 Against *Aspergillus parasiticus*. Probiotics and Antimicrobial Proteins, 11: 55–64.

https://doi.org/10.17221/175/2020-CJFS

Rouse S., Harnett D., Vaughan A., Sinderen D.V. (2008): Lactic acid bacteria with potential to eliminate fungal spoilage in foods. Journal of Applied Microbiology, 104: 915–923.

Russo P., Arena M. P., Fiocco D., Capozzi V., Drider D., Spano G. (2017): *Lactobacillus plantarum* with broad antifungal activity: A promising approach to increase safety and shelf-life of cereal-based products. International Journal of Food Microbiology, 247: 48–54.

Sangmanee P., Hongpattarakere T. (2014): Inhibitory of multiple antifungal components produced by *Lactobacillus plantarum* K35 on growth, aflatoxin production and ultrastructure alterations of *Aspergillus flavus* and *Aspergillus parasiticus*. Food Control, 40: 224–233.

Schnürer J., Magnusson J. (2005): Antifungal lactic acid bacteria as biopreservatives. Trends in Food Science & Technology, 16: 70–78.

Sevgi E., Ignatova-Ivanova T. (2015): Antifungal activity of lactic acid bacteria, isolated from Bulgarian wheat and rye flour. Journal of Life Sciences, 9: 1–6.

Ström K., Sjögren J., Broberg A., Schnürer J. (2002): *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo (L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid. Applied and Environmental Microbiology, 68: 4322–4327.

Suhr K.I., Nielsen P.V. (2004): Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. International Journal of Food Microbiology, 95: 67–78.

Walker D. K., Gilliland S. E. (1993): Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. Journal of Dairy Science, 76: 956–961.

Received: July 6, 2020
Accepted: September 21, 2020