Role of thiols and ascladiol production in patulin degradation by lactobacilli

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Running title: Patulin degradation by lactobacilli

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Significance and impact of the study

Patulin remains a safety concern in the apple processing industry. The low patulin contamination observed in (hard) ciders can be attributed to fermentation process. Lactic acid bacteria have the potential to reduce levels of patulin in foods, but the mechanisms for this activity remain poorly understood. This study expands on the knowledge of potential mechanisms by characterizing the biotransformation of patulin to ascladiol across different lactobacilli and documenting the contribution of thiol formation by lactobacilli in reduction of patulin levels.

Abstract

Patulin is a mycotoxin contaminant in various foods with apple products being its major dietary source. Yeast can reduce patulin levels during fermentation via biotransformation and thiol-adduct formation, with the ability of patulin to react with thiols being well known. Conversion of patulin to ascladiol by lactobacilli has been sparsely reported, while the contribution of thiols in reduction of patulin levels by lactobacilli remains undocumented. In this study, 11 strains of lactobacilli were screened for ascladiol formation in apple juice fermentation. Highest bioconversion was obtained for *Lactiplantibacillus plantarum* strains followed by *Levilactobacillus brevis* TMW1.465. Ascladiol production was also detected in several other lactobacilli species albeit in trace amounts. Reduction in patulin levels by *Fructilactobacillus sanfranciscensis* DMS 20451 and its glutathione reductase (Δ*gshR*) negative mutant was also assayed to determine the contribution of thiols. The hydrocinnamic acid reductase of *Furfurilactobacillus milii* did not contribute to reduction of patulin levels. In conclusion, this study demonstrated the potential of various lactobacilli in reduction of patulin levels via biotransformation of patulin to ascladiol, while also providing evidence for the role of thiol formation by lactobacilli and its presence in reducing patulin levels during fermentation.

41 **Keywords:** *Lactobacillus*; mycotoxins, patulin, phenolic acid reductase; glutathione reductase.

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Introduction

Patulin is a heat stable mycotoxin produced by over 60 species of fungi including *Penicillium*, *Aspergillus* and *Byssochlamys*. *Penicillium expansum* is regarded as main producer of patulin in food (Moake, Padilla-Zakour & Worobo, 2005; Wright, 2015). The European Food Safety Authority (EFSA) allows for a maximum of 50 µg l^{-1} patulin in fruit juices, (hard) ciders and fermented drinks containing apple juice and 25 µg kg^{-1} for solid apple products and puree (EFSA 2006). The word cider refers both to unfiltered, fresh apple juice (U.S.) or to alcoholic fermented apple juice (U.K.); in this communication, the word cider is used to refer to alcoholic fermented apple juice. Patulin levels as high as 2.7 mg l^{-1} have been reported in commercial apple juice but patulin levels are reduced by 90 – 99% by yeast fermentation (Stinson *et al.*, 1978; Harris, Bobe & Bourquin, 2009; Ioi *et al.*, 2017). Apart from pome fruits, patulin contamination can also occur in vegetables, cereals, cheeses, and shellfish, but their contribution to chronic toxicity may be insignificant compared to patulin intake via apple products (Wright, 2015).

Chronic patulin intake has been associated with various gastrointestinal symptoms such as nausea, abdominal pain, and diarrhea (Fung & Clark, 2004; Mandappa, Basavaraj & Manonmani, 2018). Animal studies have shown DNA damage in liver, hippocampus, and kidneys along with increased cardiotoxicity due to acute consumption of patulin at a level of 1.0–3.75 mg kg^{-1}) (de Melo *et al.*, 2012; Boussabbeh *et al.*, 2015). Patulin forms adducts with thiols resulting in reduction of cellular glutathione and generating oxidative stress (Fliege & Metzler, 2000). It also reacts with nucleotide bases leading to DNA damage and genotoxicity (Pfenning *et al.*, 2016). Its possible presence in several foods and agricultural products necessitates its accurate detection and detoxification to keep its concentration below the thresholds set by regulatory agencies.

Implementation of stringent controls in post harvest processing can reduce the risk of mycotoxin accumulation (Errampalli, 2014; Ioi *et al.*, 2017). Pasteurization, microfiltration, clarification and radiation have been shown to reduce patulin levels effectively alone or in combination with other processing steps (Diao *et al.*, 2018). The use of food fermenting yeasts and lactic acid bacteria has been proposed as an alternative for reduction of patulin levels and most studies focussed on adsorption to microbial cells as a means of reducing the mycotoxin levels. Live and heat inactivated cells of yeasts or lactic acid bacteria reduced the patulin content by more than 90% but the efficacy varied for different initial concentrations of patulin and the biomass of the microbial strains (Zoghi *et al.*, 2017; Zheng *et al.*, 2020; Bahati *et al.*, 2021; Lai *et al.*, 2022).

Reduction in patulin levels via biodegradation has also been reported by many yeasts including *Saccharomyces cerevisiae, Candida guilliermondii* and *Rhodosporidium kratochvilovae*, which produce ascladiol and / or desoxypatulinic acid from patulin (Castoria *et al.*, 2011; Zhong *et al.*, 2021; Luo *et al.*, 2022). Among lactic acid bacteria, only ascladiol production by few strains of *Lp. plantarum* has been reported (Hawar *et al.*, 2013; Wei *et al.*, 2020). In addition, patulin forms adducts with thiol compounds (Fliege & Metzler, 2000; Schebb *et al.*, 2009) but the contribution of thiol accumulation by lactic acid bacteria (Jänsch *et al.*, 2007) in reduction of patulin levels remains unknown. This study aimed to better understand the mechanisms of reduction of patulin levels of lactobacilli and in particular to assess the relevance of bioconversion, formation of thiol-adducts, and adsorption to the cell wall. Therefore, 11 strains of lactobacilli belonging to 6 different genera were screened for patulin adsorption and biodegradation in apple juice. The contribution of thiols in reduction of patulin levels was assayed by comparison of cultures of *Fructilactobacillus sanfranciscensis* DMS 20451 and its glutathione reductase (Δ*gshR*) negative mutant.

Materials and Methods

Bacterial strains and growth conditions

All the strains used in this study were subcultured from -80°C stocks and grown in modified de Man, Rogosa and Sharpe (mMRS) media (Zhao & Gänzle, 2018). *Lactiplantibacillus plantarum* TMW1.460 (Ulmer, Ganzle & Vogel, 2000), *Lp. plantarum* LA1 (Ripari, Bai & Gänzle, 2019), *Apilactobacillus kunkeei* DSM 12361, *Levilactobacillus brevis* TMW1.465 (Behr, Gänzle & Vogel, 2006), *Levilactobacillus hammesii* DSM 16381 (Valcheva *et al.*, 2005), *Ff. milii* FUA3583 (Pswarayi & Gänzle, 2019; Simpson *et al.*, 2022), *Furfurilactobacillus milii* C5 (Ripari *et al.*, 2019), *Fructilactobacillus sanfranciscensis* DSM 20451 and *Limosilactobacillus fermentum* FUA3590 (Pswarayi & Gänzle, 2019) were grown under microaerophilic conditions at 30°C while *Limosilactoabcillus reuteri* DSM 20016 was incubated at 37°C. *Fl. sanfranciscensis* DSM 20451 Δ*gshR* (Jänsch *et al.*, 2007) lacking glutathione reductase activity was also used having identical growth conditions as the wild type strain with the addition of erythromycin (10µg ml^{-1}).

Chemicals

Patulin (98% purity) and erythromycin (Em) were purchased from Millipore Sigma (St. Louis, MO, USA). Apple juice was purchased from a local supermarket. Media components used to make mMRS were obtained from BD (Sparks, MD, USA) while remaining components were obtained from Millipore Sigma (St. Louis, MO, USA).

Fermentations in mMRS and apple juice in the presence of patulin

Overnight cultures were washed with sterile water and resuspended in fresh mMRS and mMRS without cysteine (Cy) media. Patulin was added to mMRS and mMRS w/o Cy for a final concentration of 50 mg l^{-1} and inoculated with 10% of respective resuspended overnight cultures.

Samples were then incubated at 30°C for 24 h. Uninoculated media with and without patulin were used as controls.

Apple juice was adjusted to a pH of 5.8 using of 5M sodium hydroxide (NaOH) followed by addition of patulin. Apple juice (900 µL) was then inoculated with 100 µL of washed overnight cultures resuspended in sterile water to obtain a final patulin concentration of 50 mg l^{-1}. For preparation of concentrated inoculums, 1ml of cells were centrifuged at 8600g for 10 min and resuspended in 100 µL of sterile water. Heat inactivated controls were prepared by treating washed cells at 60°C for 30 min followed by addition to apple juice patulin samples. Uninoculated apple juice with and without patulin were used as controls. The patulin levels of unsupplemented apple juice were below the detection limit of the HPLC-UV assay used in this study, 1 mg L^{-1}. All apple juice samples were incubated at 30°C for 48 h except *Lm. reuteri* DSM 20016 samples which were incubated at 37°C. Cultures in mMRS and pH adjusted apple juice were routinely monitored by determination of viable cell counts and by measurement of the pH to verify strain identity and metabolic activity.

Quantification of patulin and its metabolites using HPLC and LC/MS

Samples were centrifuged at 8000g for 10 min for removal of cells. Supernatant was acidified using hydrochloric acid (HCl) followed by addition of 500 µL ethyl acetate. Solvent extraction was performed twice, and extracts were mixed for analysis (Ripari *et al.*, 2019). Samples were run on an Agilent 1200 series HPLC system equipped with a reverse phase XDB C-18 column (4.6 by 150 mm; 5 µm) using the protocol established by Gaur *et al.*, 2020. Quantification was performed using a UV detector at 280 nm along with a patulin external standard.

RP-HPLC-MS was performed using an Agilent 1200 SL HPLC system for the identification patulin degradation product. Samples were run on Phenomenex Luna omega C18 column (50 by 2.1 mm; 1.6 µm) with trap cartridge at 50°C. Solvents consisted of 0.1% formic acid in water (A) and 0.1% formic acid in 100% acetonitrile. The following gradient was used at a flow rate of 0.5 ml min^{-1}- isocratic at 1% B (0-1.5 min), 1% to 95% B (1.5-5.5 min), isocratic at 95% B (5.5-6.5 min) and 95 to 1% B (6.5-7 min). Analytes were detected at 280 nm using a diode array detector. Mass spectra were acquired using an Agilent 6220 Accurate-Mass TOF HPLC/MS system (Santa Clara, CA, USA) equipped with a dual sprayer electrospray ionization source.

Statistical Analysis

Data was analyzed using one way analysis of variance (ANOVA) followed by Holm-Sidak or Tukey post hoc test. Apple juice data between live and heat inactivated cultures for each strain was analyzed using t-test in using SigmaPlot 12.5 (Systat Software Inc.).

Results and Discussion

Reduction of patulin levels and metabolite formation by lactobacilli

The conversion of patulin by lactobacilli was assayed in pH-adjusted apple juice. To account for the reduction of patulin levels via adsorption, heat inactivated cultures of lactobacilli were also incubated in similar conditions and compared to viable cells. Patulin levels in spiked apple juice were lower than the expected 50 mg l^{-1} (Figure 1), either due to errors in liquid handling or because patulin reacts with other components in apple juice.

Lm. reuteri DSM 20016 was the only strain that significantly reduced patulin levels after addition of heat inactivated cultures to the pH adjusted apple juice, indicating high adsorption of patulin (Figure 1). Reduction in patulin levels by adsorption is dependent on initial concentration of toxin,

cell density and pH of the solution (Fuchs *et al.*, 2008). *Lp. plantarum* ATCC 8014 and *L. kefiranofaciens* JKSP109 were shown to remove >90% of 100 µg l^{-1} patulin from supplemented apple juice inoculated with > log 10 CFU ml^{-1} cells at pH <4 (Zoghi *et al.*, 2019; Bahati *et al.*, 2021). The lower cell density (7-8 log CFU ml^{-1}) and high concentration of patulin (50 mg l^{-1}) used in this study can explain the lack of patulin removal by heat inactivated cultures via adsorption. Increasing the microbial load by 10 times had a positive influence on patulin reduction via adsorption (Data not shown).

Patulin levels in apple juice inoculated with live cultures of *Lv. brevis* TMW1.465, *Ff. milii* C5, and *Lm. fermentum* FUA3589 differed significantly from the patulin levels in apple juice supplemented with and heat-inactivated cells of the same strains but the strains did not reduce patulin levels relative to the uninoculated control (Figure 1). Cultures of *Lp. plantarum* TMW1.460 were the only cultures which substantially reduced patulin concentrations in comparison to both uninoculated controls and heat inactivated culture samples (Figure 1), suggesting degradation of patulin as the major route of detoxification. Of the species used in this study, *Lp. plantarum* is also among the most acid resistant organisms that exhibits growth and metabolic activity in fermenting fruit juices or mashes at a pH of 3.5, i.e. the pH of apple juice without pH-adjustment (Dueñas *et al.*, 1994; Iorizzo *et al.*, 2016).

Identification of ascladiol production by lactobacilli

The area of the putative patulin metabolite was highest for *Lp. plantarum* TMW1.460 and *Lv. brevis* TMW1.465 (data not shown). Biotransformation of patulin to ascladiol was first reported in *Saccharomyces cerevisiae* with both isomers *(E)*-ascladiol and *(Z)*-ascladiol found in anerobic conditions (Moss & Long, 2002). *(E)*-Ascladiol was the predominant product while its conversion to *(Z)*-ascladiol is thought to be the result of a non-enzymatic reaction (Moss & Long, 2002).

Gluconobacter oxydans and *Lp. plantarum* also degraded patulin by conversion to ascladiol (Ricelli *et al.*, 2007; Hawar *et al.*, 2013). For identification of the degradation peak observed in several lactobacilli samples in both mMRS and apple juice media, samples from cultures of *Lp. plantarum* TMW1.460 were analyzed by LC/MS. The peak at *m/z* 157.0494 in positive ion mode matches the molecular ion peak of ascladiol + H^+. In addition, the characteristic fragments at *m/z* 131 and *m/z* 114 were present (Hawar *et al.*, 2013; Wei *et al.*, 2020) (Figure S1). The compound was tentatively identified as ascladiol which may be the result of an ether bond cleavage in patulin. To confirm which other strains had the ability to produce ascladiol, apple juice fermentation samples for remaining lactobacilli were also analyzed using LC-MS. Ascladiol was present in culture supernatants of all strains except *Ap. kunkeei* DSM 16341 and *Fl. sanfranciscensis* DSM 20451 after 48 h of incubation (Table 1). To check the influence of higher initial microbial concentration on ascladiol production, apple juice medium inoculated with 10 times (10X) concentrated cell suspensions were also analyzed. The relative peak area of the ascladiol peak increased for all the strains except *Lv. hammesii* DSM 12361. Interestingly, ascladiol formation was also detected in *Lm. reuteri* DSM 20016 and *Fl. sanfranciscensis* DSM 20451 upon increasing the cell density. The peak area corresponding to ascladiol was highest in *Lp. plantarum* TMW1.460, followed by *Lv. brevis* TMW1.465.

To date, the conversion of patulin to ascladiol in lactobacilli has been reported only for few strains of *Lp. plantarum* (Hawar *et al.*, 2013; Wei *et al.*, 2020). The present study detected low levels of ascladiol also in culture supernatants of *Lv. brevis* TMW1.465, *Lv. hammesii* DMS12361, *Ff. milii* C5, *Ff. milii* FUA3583, *Lm. reuteri* DSM 20016, *Lm. fermentum* FUA3589 and *Fl. sanfranciscensis* DSM 20451. Interestingly, both hop resistant beer spoiling strains used in this study namely, *Lp. plantarum* TMW1.460 and *Lv. brevis* TMW1.465 showed high ascladiol

198 production. Because this study did not systematically quantify growth and metabolic activity in
199 pH-adjusted apple juice, this result may reflect a superior ability to produce ascladiol, or a
200 generally higher metabolic activity in apple juice when compared to other lactobacilli.

201 We additionally hypothesized that Par2, an uncharacterized homolog of the hydroxycinnamic acid
202 Par1 (Gaur *et al.*, 2020), may be involved in reduction of ascladiol to hydroascladiol. However,
203 incubation with *Ff. milii* FUA3583 and its isogenic mutants lacking genes coding for Par enzymes
204 showed identical patulin profile with presence of hydroascladiol unconfirmed (Table S1).

205 Ascladiol production by other yeast species such as *Meyerozyma guilliermondii*, *Sporobolomyces*
206 sp. and *Candida guilliermondii* has been attributed to an inducible short-chain dehydrogenase
207 (Chen *et al.*, 2017; Ianiri *et al.*, 2017; Luo *et al.*, 2022). Recently, Xing *et al.*, 2021 heterologously
208 expressed and characterized CgSDR from *C. guilliermondii* 2.63 responsible for converting patulin
209 to ascladiol. Lactobacilli possess numerous putative alcohol dehydrogenases with unknown
210 substrate specificities (Liu *et al.*, 2008) which, in analogy to yeasts, also may contribute to
211 ascladiol. The production of ascladiol might also be result of dehydrogenase enzymes displaying
212 moonlighting activity on patulin (Jeffery, 2018). Another possible patulin metabolite includes
213 desoxypatulinic acid, which is produced by some yeasts such as *Rhodosporidium kratochvilovae*
214 and *Rhodotorula mucilaginosa* (Castoria *et al.*, 2011; Li *et al.*, 2019), but its production by
215 lactobacilli has not been reported.

216 It is generally believed that patulin conversion to ascladiol reduces toxicity but the data on the
217 toxicity of dietary ascladiol in humans remains limited (Tannous *et al.*, 2017). A histological study
218 on pig intestinal tissue showed ascladiol to be non-toxic (Maidana *et al.*, 2016). Ascladiol has low
219 to no toxicity at concentrations of less than 5 mg l^{-1} on some human cell lines including human
220 colon carcinoma (Caco-2), human hepatoma (HepG2) and human esophageal epithelial cells (Het-

1a) (Tannous *et al.*, 2017; Zheng *et al.*, 2018; Yang *et al.*, 2021). The conversion of patulin to ascladiol by lactobacilli thus likely reduces the toxicity.

Effect of glutathione reductase and thiols in reduction of patulin levels

Heterofermentative lactobacilli but not homofermentative lactobacilli accumulate thiols during growth in food fermentations (Jänsch *et al.*, 2007; Gänzle, 2015). To assess the role of thiols in reduction of patulin levels, *Fl. sanfranciscensis* DSM20451 and its glutathione reductase mutant strain Δ*gshR* were incubated in the presence/ absence of cysteine in mMRS broth containing patulin (50 mg l^{-1}) for 24 h at 30°C. In the presence of cysteine, the wild type strain significantly reduced the patulin concentration in comparison to the Δ*gshR* mutant (Figure 2). Patulin can undergo electrophilic reactions and has been shown to form a variety of glutathione adducts in the presence of glutathione (Fliege & Metzler, 2000; Schebb *et al.*, 2009). Concentrations of glutathione and cysteine were found to be significantly reduced in patulin treated *Saccharomyces cerevisiae* in comparison to untreated control (Shao, Zhou & McGarvey, 2012).

In this study, the presence of cysteine in uninoculated controls did not affect the patulin concentrations significantly. Lack of cysteine in the media affected patulin levels. Addition of cysteine resulted in reduced patulin concentrations in media inoculated with *Fl. sanfranciscensis* when compared to the cultures without addition of cysteine. The wild type strain reduced greater patulin concentrations to a greater extent than the Δ*gshR* mutant irrespective of cysteine addition. No significant differences were observed between Δ*gshR* mutant and its corresponding controls in both the conditions. In contrast, the wild type strain significantly reduced the patulin concentration in the presence of cysteine compared to the uninoculated control. Presence of cysteine in acidic conditions has been shown to reduce patulin levels at temperatures of 90°C and above where patulin is shown to have high relative stability (Diao *et al.*, 2021). Liu *et al.*, 2019 created a cysteine

based synthetic metal-organic framework adsorbent which effectively removed 4.38 μg of patulin per mg of adsorbent from apple juice.

None of the *Fl. sanfranciscensis* strains altered patulin concentrations in pH-adjusted apple juice relative to uninoculated controls (Figure 1). *Fl. sanfranciscensis* does not perform well in apple juice in the absence of appropriate energy source for metabolism, which was evident by Δ*gshR* mutant's struggle for growth with a high final pH (5.56 ± 0.09) obtained after incubation. To overcome the growth deficit, samples inoculated with concentrated (10X) cultures were also analyzed. Increasing the initial microbial counts allowed detection of low levels of ascladiol but did not reduce patulin concentration after incubation of *Fl. sanfranciscensis* strains in apple juice (Figure S2). Although data for pH-adjusted apple juice is unavailable, the higher reduction of patulin levels in mMRS obtained in the presence of glutathione reductase and cysteine provides evidence for role of thiols in reducing patulin levels by lactic acid bacteria.

Can lactobacilli effectively reduce patulin levels in apple products?

Liquorilactobacilli such as *Lq. mali* and *Lq. sicerae* are frequently isolated from fermented apple ciders (Carr & Davies, 1970; Puertas *et al.*, 2014). Other lactobacilli such as *Lp. plantarum* and *Lv. brevis* are also part of dominant microbiota in traditional cider production (Dueñas *et al.*, 1994). In our study, both *Lp. plantarum* and *Lv. brevis* strains converted significant amounts of patulin to ascladiol in apple juice. Many other lactobacilli strains tested also produced trace amounts of ascladiol. Growth of different strains of *P. expansum* on apple fruits results in patulin concentrations ranging from less than 1 to 125 mg patulin kg^{-1} (Sommer, Buchanan & Fortlage, 1974) but the concentration of patulin used in this study is 1000 times higher than the maximum permitted limit and very unlikely to be present in actual apple products (EFSA 2006). This might

enable the small significant differences observed due to thiols and trace ascladiol production being relevant in reduction of patulin levels.

The low risk of patulin contamination in cider production can be attributed to fermentation, with yeast being the major contributor in comparison to lactobacilli (Cousin *et al.*, 2017). Fermentation with yeast lead to a 99% reduction of 15 mg l^{-1} patulin concentration in spiked apple juice (Stinson *et al.*, 1978). A recent study by Zhong *et al.*, 2021 showed that ascladiol producing *Saccharomyces cerevisiae* S288C also converts patulin into other metabolites including glutathione adducts such as c-GSH-PAT and l-GSH-PAT. The concentrations of ascladiol and desoxypatulinic acid obtained (<10 %) do not correspond with the concentration of patulin reduced by yeasts (Ianiri *et al.*, 2013; Zhong *et al.*, 2021). Also, ascladiol formation was limited to fermentation, with cell lysate predominantly reducing paulin levels via GSH adduct formation with heat treatment having no significant effect in reduction of patulin levels (Zhong *et al.*, 2021). The production of other unknown metabolites remains possible, but in yeasts, thiol adduct formation likely is the predominant route of detoxification. Patulin thiol adducts have been shown to be significantly less toxic than their parent mycotoxin but studies regarding effects of GSH-patulin adducts consumption and safety remain limited (Lindroth & Von Wright, 1978; Diao *et al.*, 2022).

Patulin contamination in other foods remains possible. A survey of semi-hard cheeses revealed the presence of 15 to 460 μg kg^{-1} of patulin, but most of it was limited to the rind (Pattono *et al.*, 2013). Based on quantity of dietary intake and contamination levels present, the contribution of other foods to chronic patulin toxicity in humans remains unlikely (Cunha *et al.*, 2014; Dobson, 2017; Ji *et al.*, 2017). Furthermore, lactobacilli may contribute to removal of patulin during food fermentations especially in the absence of yeasts. Based on limited ascladiol production observed

in this study, thiol adduct formation may be the main contributor except for strains showing very high ascladiol production such as strains of *Lp. plantarum*.

Application of lactobacilli as adsorbents may not be a practical alternative to existing physical and processing steps involved in the production of apple products. They possess a risk of producing metabolites which can alter sensory attributes, are required in large amounts for use in industrial scale and their efficiency is highly sensitive to environmental factors (Diao *et al.*, 2018). Use of well characterized synthetic adsorbents and clarifying agents might be more promising given their consistent performance along with easier integration in the processing plants (Liu *et al.*, 2019a).

In conclusion, lactobacilli have the potential to effectively reduce patulin levels in pome fruit products and fermentations. They can do this by enzymatic conversion of patulin to ascladiol and/or by maintaining a supply of thiols facilitating patulin adduct formation.

Acknowledgements

We thank Béla Reiz and Dr. Randy Whittal for their technical expertise and assistance in LC/MS analysis. The Natural Sciences and Engineering Council of Canada is acknowledged for funding under the Discovery Grant program. Michael Gänzle acknowledges support from the Canada Research Chairs program.

Conflict of Interest

Authors report no conflict of interest declared.

Data Availability Statement

Data available within the article or its supplementary materials

Author Contribution Statement

Conceptualization, MGG; Methodology , GG; Investigation, GG; Writing, GG; Writing – review & Editing, GG and MGG; Visualization, GG; Funding acquisition , MGG

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Figure legends

Figure 1. Influence of live and heat inactivated lactobacilli strains on patulin concentration after 48 h apple juice fermentation at 30°C. Bars with * represents significant differences ($P<0.05$) with respect to uninoculated control (White bar). Bars with # represents significant differences between live and heat inactivated cultures for each strain. Data is represented as means ± SD of three independent experiments.

Figure 2. Contribution of thiols in patulin reduction by *Fructilactobacillus sanfranciscensis* DSM 20451. Strains were incubated for 24 h in mMRS medium containing patulin with or without cysteine (Cys, 0.5 g l^{-1}) at 30°C. WT- Wild type strain; Δ*gshR*- Mutant strain with inactivated glutathione reductase gene. Bars with different letters are significantly different ($P<0.05$). Data is represented as means ± SD for three replicates.

Table 1. Summary of ascladiol production by lactobacilli strains. Different symbols represent values of relative peak area obtained for ascladiol after 48 h fermentation in apple juice. Presence of ascladiol was confirmed using LC/MS. ++: >25 %, +: >10 %**,** ±: >2 %, -: Not detected, X- Not determined.

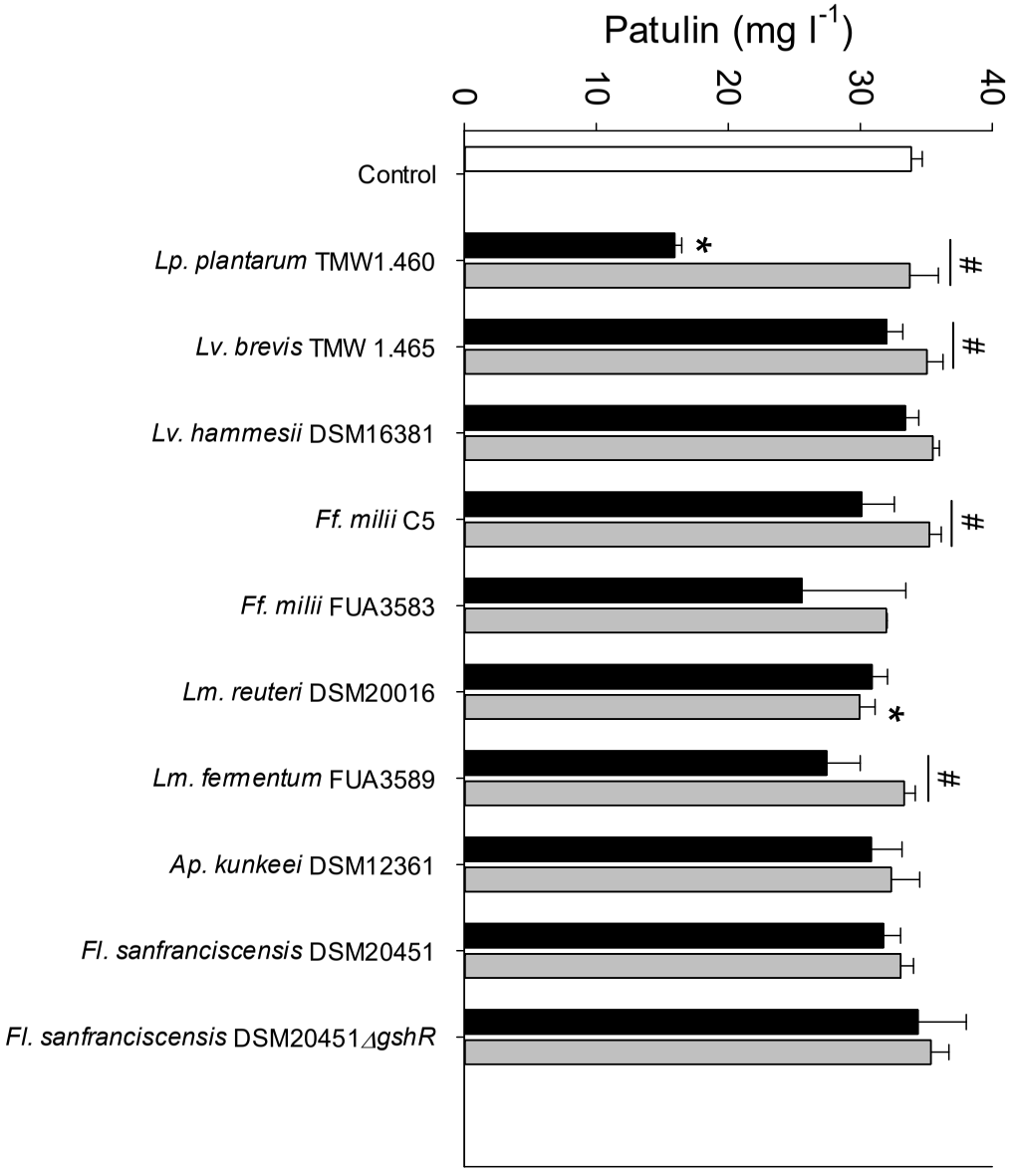
Strains	**Inoculum Concentration**	
	1X	10X
Lactiplantibacillus plantarum TMW1.460	++	
Lactiplantibacillus plantarum LA1	++	
Levilactobacillus brevis TMW1.465	±	+
Levilactobacillus hammesii DMS 12361	±	
Furfurilactobacillus milii C5	±	
Furfurilactobacillus milii FUA3583	±	
Limosilactobacillus reuteri DSM 20016	X	±
Limosilactobacillus fermentum FUA3589	±	
Apilactobacillus kunkeei DSM 16341	-	-
Fructilactobacillus sanfranciscensis DSM 20451	-	±
Fructilactobacillus sanfranciscensis DSM 20451 Δ*gshR*	-	-

Online Supporting Information.

Figure S1. HPLC-UV and LC-MS/MS analysis of patulin spiked apple juice fermented with *Lp. plantarum* TMW1.460.

Figure S2. Influence of *Fructilactobacillus sanfranciscensis* on patulin concentration in apple juice.

Table S1. Patulin metabolism by *Furfurilactobacillus milii* FUA3583 and isogenic mutants phenolic acid reductase mutants.


Patulin (mg l-1)
0
10
20
30
40
Control
Lp. plantarum TMW1.460
Lv. brevis TMW 1.465
Lv. hammesii DSM16381
Ff. milii C5
Ff. milii FUA3583
Lm. reuteri DSM20016
Lm. fermentum FUA3589
Ap. kunkeei DSM12361
Fl. sanfranciscensis DSM20451
Fl. sanfranciscensis DSM20451ΔgshR
*
#
#
#
*
#

Patulin mg l-1
25
20
15
10
5
0
b
ab
c
b
b
a
mMRS
mMRS w/o Cys
WT
ΔgshR
WT w/o Cys
ΔgshR w/o Cys

Online supplementary material to

Role of thiols and ascladiol production in patulin degradation by lactobacilli

Gautam Gaur and Michael G. Gänzle

Figure S1. HPLC-UV and LC-MS/MS analysis of patulin spiked apple juice fermented with *Lp. plantarum* TMW1.460.

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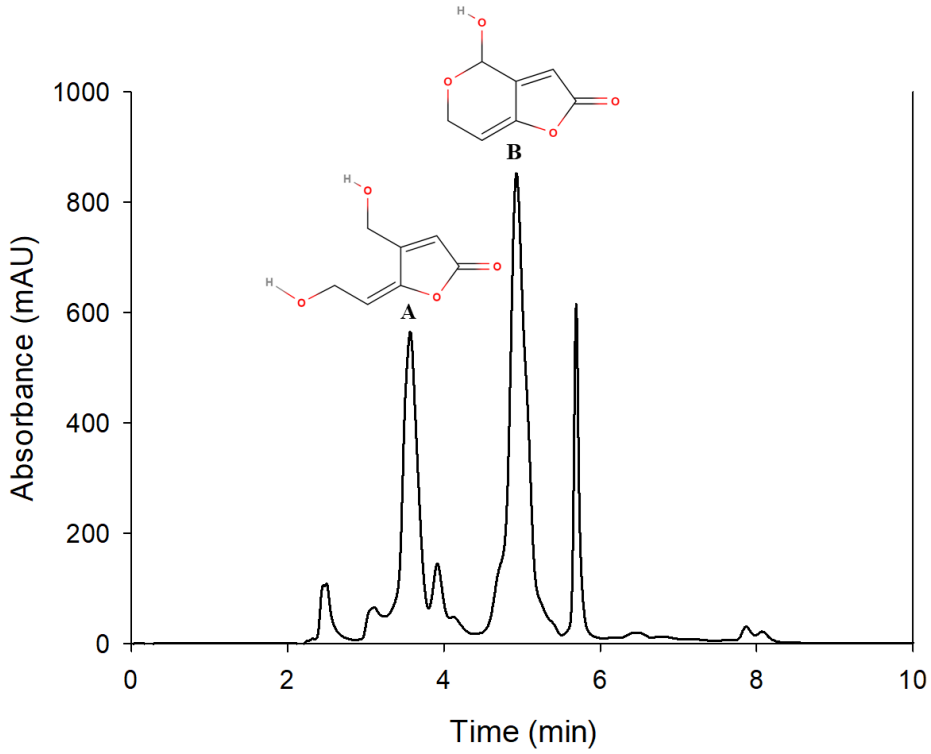
a)



b)

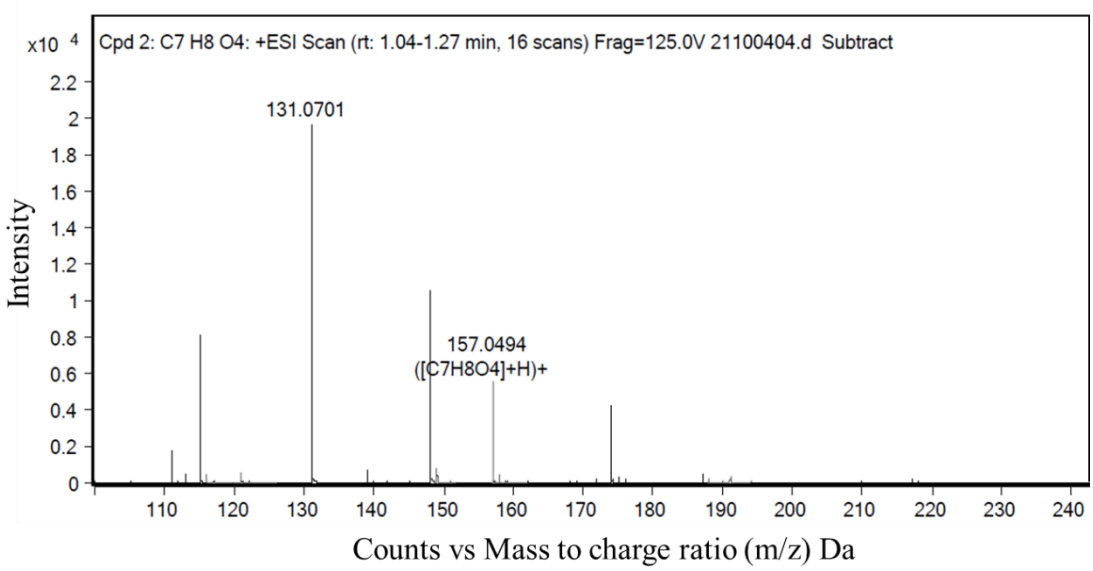


Figure S1. a) HPLC-UV chromatogram (280 nm) of patulin spiked apple juice fermented with live *Lp. plantarum* TMW1.460 culture for 48 h at 30°C. Peak A) ascladiol; peak B) Patulin. b) LC/MS spectrum of peak A) identified as ascladiol (m/z- 157.0494).

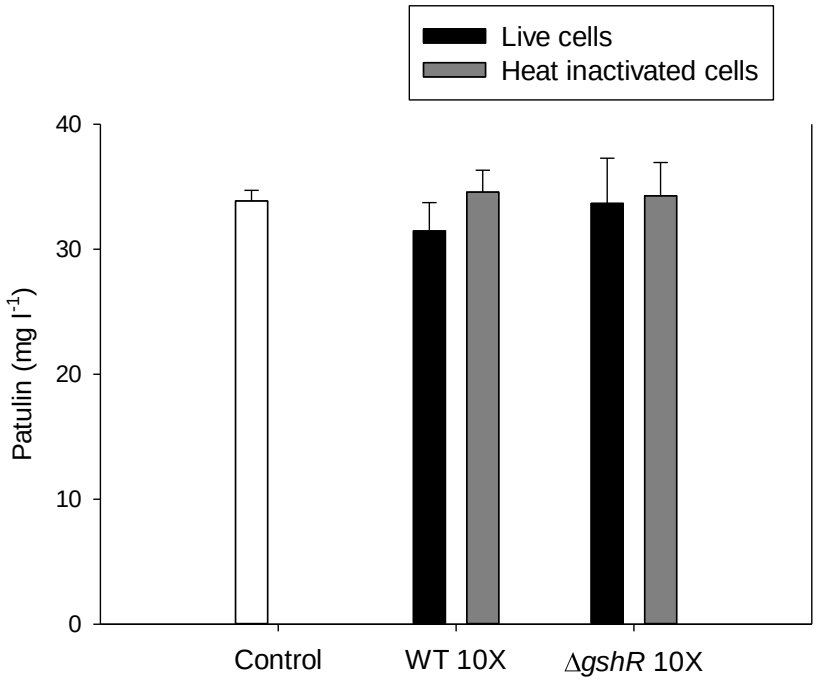


Figure S2. Influence of live and heat inactivated *Fructilactobacillis sanfranciscensis* DSM 20451 cultures with concentrated inoculums (10X) on patulin concentration after 48 h apple juice fermentation at 30°C. WT- Wild type strain; Δ*gshR*- Mutant strain with inactivated glutathione reductase gene. Data is represented as means ± SD for three independent experiments.

Table S1. Patulin metabolism by *Furfurilactobacillus milii* FUA3583 and isogenic mutants in mMRS broth. *par1*- Phenolic acid reductase, *par2*- uncharacterized homolog of Par1. (±) represents presence in trace amounts; (-): not detected.

Strain	Ascladiol	Hydro-ascladiol
Furfurilactobacillus milii FUA3583	±	-
Furfurilactobacillus milii FUA3583 Δ*par2*	±	-
Furfurilactobacillus milii FUA3583 Δ*par1*Δ*par2*	±	-