Antifungal Mechanisms and Application of Lactic Acid Bacteria in Bakery Products: A Review

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Bakery products are nutritious, but they are susceptible to fungal contamination, which leads to a decline in quality and safety. Chemical preservatives are often used to extend the shelf-life of bakery products, but long-term consumption of these preservatives may increase the risk of chronic diseases. Consumers increasingly demand food with fewer chemical preservatives. The application of lactic acid bacteria (LAB) as a novel biological preservative not only prolongs the shelf-life of bakery products but also improves the baking properties of bakery products. This review summarizes different types and action mechanisms of antifungal compounds produced by LAB, factors affecting the production of antifungal compounds, and the effects of antifungal LAB on bakery products, providing a reference for future applications of antifungal LAB in bakery products.

Keywords: lactic acid bacteria, antifungal mechanisms, bakery products, biological preservative, fungal contamination

INTRODUCTION

Bakery products are among the most popular foods consumed daily by people throughout the world (ReportLinker, 2020). However, bakery products are susceptible to fungal contamination that greatly reduces their shelf-life, resulting in food waste and economic loss. Fungal contamination can also become a serious food safety issue due to the potential production and ingestion of toxic aflatoxins and other mycotoxins (Mahato et al., 2021). Inhibition of fungal growth on bakery products therefore offers significant economic and health benefits. The primary methods of inhibition that are being explored to extend the shelf-life of bakery products currently focus on physical methods (radio frequency sterilization, microwave sterilization, drying, pulsed-light, and low-pressure mercury lamp treatment) and chemical preservatives (calcium propionate, sorbate, benzoates, EDTA, nitrites, and sulfites; Salaheen et al., 2014). Although physical methods are better at maintaining taste, they simultaneously destroy the nutritional value of bakery products and are often costly. Calcium propionate and other chemical preservatives can be added directly to bakery products; however, long-term consumption of these preservatives may increase the risk of chronic disease (Qian et al., 2021). Food companies are interested in reducing the use of chemical preservatives because of the need of consumers for preservative-free foods (Sadeghi et al., 2019). Biological preservatives, on the other hand, are more consumer-friendly, ecologically sustainable, and have prospectively broad applications in controlling fungal contamination. Lactic acid bacteria (LAB) have gained attention as a potential biological preservative option since they are generally recognized as safe, and produce metabolites that
can inhibit fungal growth. For example, Korcar et al. (2021) found that Pedicoccus pentosaceus MB33 and Weissella cibaria CM32 exhibited high antifungal activity, enhancing the shelf-life of emmer bread. LAB also improve baking properties of bakery products including texture, specific volume, and flavors of bakery products (Ma et al., 2021). Several antifungal compounds produced by Leuconostoc citreum HO12 and Weissella koreensis HO20 improved the flavor and texture of bakery products (Choi et al., 2012). Therefore, investigating the application of antifungal LAB in bakery products has substantial value. While there are reviews on the application of antifungal LAB in foods, and mechanisms of antifungal substances, few of them have comprehensively focused on the application of LAB as biopreservatives in bakery products, and the antifungal mechanism of exopolysaccharides (EPS; Crowley et al., 2013a; Chen et al., 2021b; Nasrollahzadeh et al., 2022). The present review seeks to present an update on the antifungal compounds produced by LAB and their mechanism of action toward target fungi, factors affecting the production of antifungal compounds by LAB, and the application of LAB in bakery products.

HEALTH PROBLEMS CAUSED BY FUNGAL CONTAMINATION

The most common fungi related to spoilage of bakery products include Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus, Candida, and Endopyrrhiza (Mohamad Asri et al., 2020). Fungal contamination not only reduces the quality of bakery products but also results in huge economic losses to both consumers and the bakery industry. In addition, some fungi (Aspergillus, Penicillium, Fusarium, etc.) may produce mycotoxins such as aflatoxin, ochratoxin, and zearalenone (Krnjaja et al., 2019; Drakopoulos et al., 2021). Aflatoxins are difuran ring toxoids mainly produced by certain strains of Aspergillus flavus and Aspergillus parasiticus (Wang et al., 2022a). Aflatoxins are carcinogenic, teratogenic, and mutagenic and can damage the liver of animals (Ben Taheur et al., 2019). Aflatoxin B1 (AFB1) is the most toxic type of aflatoxin and is a significant risk factor for the development of hepatocellular carcinoma (HCC) in humans and animals (Fang et al., 2022). Ochratoxins are mycotoxins derived from Aspergillus and Penicillium, and their toxic effects include hepatotoxicity, carcinogenicity, nephrotoxicity, immunotoxicity, teratogenicity, mutagenicity, genotoxicity, embryotoxicity, as well as testicular toxicity (Amézqueta et al., 2012; Wang et al., 2022b). Zearalenone-producing fungi are mainly Fusarium. Zearalenone causes obvious estrogenic effects in both humans and animals, as well as serious malignant alterations and lesions in the female reproductive system (Sanad et al., 2022).

OVERVIEW OF LAB

LAB are Gram-positive, non-spore-forming, facultatively anaerobic bacteria that produce a large amount of lactic acid during carbohydrate metabolism. LAB are widely distributed in nature, and include genus such as Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Tetragonococcus, Oenococcus, Weissella, and Vagococcus (Stiles and Holzapfel, 1997). LAB play a critical role in food fermentation to enhance food shelf-life (Pontonio and Rizzello, 2021). LAB induce rapid acidification of raw materials during fermentation, producing organic acids, CO₂, H₂O₂, fatty acids, antifungal peptides, volatile compounds, and other antifungal compounds that inhibit fungal growth (Sadeghi et al., 2019). Moreover, mycotoxins can be detoxified by LAB with the help of one or more mechanisms. For instance, the utilization of metabolites and enzymes produced by LAB strains, adsorption of mycotoxins by LAB, or competitive relationship between LAB and other mycotoxin producing fungi (Ahlberg et al., 2015). LAB produce a variety of proteolytic enzymes, including cell-wall proteinases, peptide transporters, and ample intracellular peptidases, which are capable of biodegradation of mycotoxins to less toxic and less harmful compounds (Bangar et al., 2021). Citric acid and other organic acids produced by LAB also have a great effect on degrading aflatoxins. The results from Mendez-Albores et al. (2008) showed that the aflatoxin reduction was more effective when adding aqueous citric acid in the extrusion-cooking process. Ademola et al. (2021) found that lactic acid fermentation also reduced aflatoxin and fumonisin levels in maize. LAB cell wall including peptidoglycan and polysaccharides can adsorb mycotoxin, which also caused the toxin removal (Shetty and Jespersen, 2006). As a biological preservative, LAB significantly reduce the usage of chemical preservatives in bakery food. In addition, LAB also produce exopolysaccharides, ethanol, and volatile flavor substances during fermentation, which bring balanced bread sensory profiles. The α-glucan-producing Lactobacillus reuteri E81 was used in bread making, which improved the rheological properties, elasticity, and microstructure of bread dough, and the texture of bread (İspirli et al., 2020). Wu et al. (2022) found that sourdough fermented with Lactococcus lactis FCP1921 had improved volatile flavor substance abundance in bread when compared with ordinary wheat bread.

ANTIFUNGAL COMPOUNDS PRODUCED BY LAB

Metabolism of carbohydrates, proteins, lipids, and amino acids by LAB produces a variety of antifungal compounds discussed further here (Figure 1).

Organic Acids

LAB produce lactic acid, acetic acid, propionic acid, citric acid, phenyllactic acid, benzoic acid, and other organic acids. Most of these organic acids have antifungal properties; however, action mechanisms of some organic acids are still unclear. Studies have shown that acetic acid's inhibition of the growth of pathogenic and spoilage bacteria and fungi may be due to its low pKa. The higher concentration of undissociated acids can traverse the cell membrane, then dissociate in the cell, resulting in acid stress (Jin et al., 2021). Relative to other
organic acids, lactic acid demonstrated weaker inhibitory effects (Crowley et al., 2013a). With increased fermentation time, the lactic acid content of cell-free supernatants was positively correlated with antifungal activity, and lactic acid frequently played a synergistic role with other organic acids to ultimately enhance antifungal activity (Russo et al., 2017). Phenolic acids isolated from quinoa dough, including derivatives of cinnamic acid (p-coumaric acid, caffeic acid, and ferulic acid) and benzoic acid (vanillic acid, 4-hydroxybenzoic acid), also showed antifungal activity (Axel et al., 2016). Phenolic acids often exist in an insoluble form, or in a more complex cross-linked polymer, which can exert antioxidant and antifungal effects (John, 2008). Furthermore, some carboxylic acids produced by LAB, including 3-phenylpropionic acid, hydroxyphenylactic acid, 3-(4-hydroxyphenyl) propionic acid, and 5-oxypyrrrolidin-2-carboxylic acid, showed inhibitory effects on *Mucor ractosum* and *Penicillium* (Leyva Salas et al., 2019). Honoré et al. (2016) isolated metabolites, such as 2-hydroxy-4-methylpentanoic acid, 2-hydroxy-3-phenylpropionic acid, and 2-hydroxy-3-(4-hydroxyphenylpropionic) propionic acid, from the cell-free supernatant of *Lacticaseibacillus paracasei*, which was positively correlated with inhibition of mold growth.

**Phenyllactic Acid**
Phenyllactic acid is derived from the catabolism of phenylalanine (Phe), which transfers its amino group to the keto acid receptor and then reduces the synthesized phenylpyruvate to phenyllactic acid (PLA) through dehydrogenase action (Vermeulen et al., 2006). PLA harbors broad spectrum antibacterial activity, which can destroy biofilm structures and inhibit the growth of pathogenic and spoilage bacteria and fungi. Dal Bello et al. (2007) isolated PLA, lactic acid, and two cyclic dipeptides from the cell-free supernatant of *Lactobacillus plantarum* FST1.7, all of which showed antifungal activity. Meanwhile, Gerez et al. (2009) obtained LAB with antifungal activity against *Aspergillus*, *Fusarium*, and *Penicillium* and also isolated PLA and acetic acid from these LAB. Le Lay et al. (2016a) detected PLA (22.04 mg/L) in the cell-free supernatant of *Lactobacillus spicheri* O15 with demonstrated antifungal activity. Like other organic acids, the antifungal activity of PLA is pH-dependent. At low pH, the undissociated form can easily pass through the cell membrane and accumulate within the cytoplasm, thereby causing loss of cell activity (Lambert and Stratford, 1999). At pH 3.5, the minimum concentration of PLA needed to inhibit 50% of the germination of different fungal spores was 0.03–0.07 mmol/L, while lactic acid and acetic acid were 80–180 and 0.9–18 mmol/L, respectively. Although PLA had higher antifungal activity than lactic acid and acetic acid, this was only true at high PLA concentrations and low pH (Gerez et al., 2009; Debonne et al., 2020). In addition, the minimum inhibitory concentration of PLA is different on different fungi. For example, PLA possesses MIC value of 2.5 mg/ml against *Aspergillus fumigatus*, in contrast to 20 mg/ml against *Aspergillus niger* (Lavermicocca et al., 2003; Ryan et al., 2011).

**Fatty Acids**
Fatty acids produced by LAB also have strong antifungal activity, and hexadecanoic acid, oleic acid, hexadecanoic acid, decanoic acid, and lauric acid isolated from antifungal LAB were confirmed...
to show inhibitory effects against *Mucor ractosum* and *Penicillium common* (Leyva Salas et al., 2019). In addition, 3-hydroxy-5-dodecenonic acid purified from *Lactobacillus plantarum* EM culture using solid-phase extraction and recycling preparative HPLC also exhibited a strong antifungal activity, with MIC of 0.21 g/L and 0.25 g/L against *Aspergillus fumigatus* and *Bacillus cereus*, respectively (Mun et al., 2019). Liang et al. (2017) extracted two kinds of fatty acids from cultures of *Lactobacillus hammesii* and *Lactiplantibacillus plantarum* and found that HUFA (unsaturated fatty acids with hydroxyl groups) effectively inhibited *Aspergillus niger* and *Penicillium roqueforti* but had poor inhibitory effects on *Candida* and other yeasts. Studies have shown that the antifungal activity of fatty acids is related to their structure; while unsaturated monohydroxy fatty acids have antifungal activity, saturated hydroxy fatty acids and unsaturated fatty acids do not (Black et al., 2013). Studies by Pohl et al. (2008) showed that the MICs of 13-HOE against *Aspergillus niger* and *Penicillium roqueforti* were 0.25 and 0.38 g/L, respectively. The MICs of 10-HOE against *Aspergillus niger* and *Penicillium roqueforti* were 0.42 and 0.38 g/L, respectively. The antifungal activity of hydroxy fatty acids might be due to their interaction with the cell membrane since distribution of hydroxy fatty acids into the fungal membrane increased membrane permeability (Sjogren et al., 2003).

\[ \text{H}_2\text{O}_2 \]

Some LAB produce \( \text{H}_2\text{O}_2 \), which has been proven to affect the growth and metabolism of foodborne pathogenic bacteria and fungi. As a strong oxidizer, \( \text{H}_2\text{O}_2 \) plunders electrons and molecules of nearby microorganisms and thus sterilizes by destroying protein molecular structure. Since LAB do not produce catalase, \( \text{H}_2\text{O}_2 \) cannot be decomposed, and therefore accumulates in the cell, preventing fungal growth (Nes et al., 1996). Bundgaard-Nielsen and Nielsen (1996) found that 3% \( \text{H}_2\text{O}_2 \) solution exhibited low antifungal activity against *Penicillium*, *Cladosporium*, *Scopulariopsis*, *Aspergillus*, and *Eurotium* but damaged conidia of seven fungal species. Martin and Maris (2012) tested the antifungal effect of \( \text{H}_2\text{O}_2 \) and 17 kinds of acids on fungi. The results showed that formic acid, acetic acid, propionic acid, oxalic acid, and lactic acid had synergistic effects with \( \text{H}_2\text{O}_2 \), resulting in stronger antifungal activity.

**Antifungal Peptides**

Antifungal peptides are the main antifungal compounds produced by LAB. Arulrajah et al. (2021) used *Lactobacillus pentosus* RK3 to ferment kenaf seeds to produce antifungal peptides, and eight cationic peptides were identified in the kenaf seed mixture, which showed inhibitory effects on *Fusarium* and *Aspergillus niger*. Among them, four peptides were shown to be similar to *Gossypium mustelinum* (cotton), two peptides corresponded to *Gossypium barbadense* (Sea-island cotton), and two were novel cationic de novo peptides. Similarly, Nionelli et al. (2020) identified nine peptides from bread hydrolysates fermented by *Lactobacillus brevis*, and these peptides prevented growth of *Penicillium roqueforti*. The inhibitory effect of antifungal peptides on fungi is mainly due to the interaction between negatively charged molecules of the fungal membrane and positively charged polypeptides, which destroys the membrane structure resulting in cell death (Rai et al., 2016). In addition, the activity of antifungal peptides produced by plant substrate fermentation was significantly affected by molecular weight, chemical structure, net charge, and hydrophobic ratio (Jakubczyk et al., 2020). Most of the antifungal peptides have low molecular weights, cationic charges, and low hydrophobicity (Muhialdin et al., 2016). Muhialdin et al. (2020) confirmed the above views, and they isolated a component targeting *Aspergillus flavus* from the cell-free supernatant of *Lactiplantibacillus plantarum* TE10 and 37 peptides identified from the isolated component—22 were cationic peptides.

Antifungal peptides also inhibited conidial germination, potentially through the inhibition of germ tube elongation after conidial wall breakdown (Puig et al., 2016). Leon et al. (2020) observed that the antifungal peptides AMPs LR14 produced by *Lactiplantibacillus plantarum* inhibit conidial germination. Fungal mycelia treated with antifungal peptides were wrinkled with no conidia detected, and the expression levels of *brlA*, a transcription factor involved in fungal meristem, were also significantly decreased.

**Volatile Compounds**

LAB produce several volatile compounds in the fermentation process, which not only improves the aroma profile but also inhibits fungal growth. Leyva Salas et al. (2019) isolated 35 volatile compounds from dairy products fermented by antifungal LAB, among which diacetyl and acetoin had antifungal activity and exhibited significant antifungal effects against *Penicillium common* and *Mucor ractosum*. Diacetyl, lactic acid, acetic acid, and acetoin were reported to be produced by catabolism of the two main carbon substrates present in dairy products, lactose and citrate (Urbach, 1995). Thus, acetoin and diacetyl with antifungal activity may be produced by lactic acid bacteria fermentation. Aunsbjerg et al. (2015) isolated diacetyl, the main volatile compound, from *Lactisasebacillus paracasei* DGCC2132, which slowed down the growth of *Penicillium* for 5 days at concentrations above 75μg/ml. The antifungal mechanisms of diacetyl may be due to the induction of ROS accumulation, which destroys the membrane structure resulting in the leakage of cellular materials and cell death (Shi and Knochel, 2021). In addition, Coda et al. (2011) found that soluble extract of *Lactiplantibacillus plantarum* by Tris-HCl buffer contained ethyl acetate and ethanol, and confirmed that the conidia germination of *Penicillium roqueforti* was completely inhibited when ethyl acetate and ethanol concentrations were 6.81 and 1.69 mg/ml, respectively.

**Cyclic Dipeptides**

Cyclic dipeptides (CDPs) are metabolites widely synthesized by cyclodipeptide synthases or non-ribosomal peptide synthetases, by both prokaryotic and eukaryotic cells. CDPs are minimal cyclic peptides formed by inner cyclization of two amino acids amides and have various bioactive properties including anticancer, immunomodulatory, and antifungal activities (Rhee, 2004).
Ström et al. (2002) reported for the first time that the production of antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-Pro) by LAB, and MIC value of cyclo(L-Phe-L-Pro) against *Penicillium roqueforti* and *Aspergillus fumigatus* was 20 mg/ml. Cyclo(L-His-L-Pro), Cyclo(L-Pro-L-Pro), Cyclo(L-Met-L-Pro), Cyclo(L-Leu-L-Pro), and Cyclo(L-Tyr-L-Pro) were isolated from the cell-free supernatant of *Lactobacillus amylovorus* LA 19280, and these cyclic dipeptides all showed antifungal activity, with MICs between 25 and 50 mg/ml against *Aspergillus fumigatus* (Ryan et al., 2011). In addition, cis-cyclo(L-Tyr-L-Pro), cis-cyclo(L-Val-L-Pro), cis-cyclo(L-Ser-L-Pro), cis-cyclo(L-Leu-L-Pro), and cis-cyclo(L-Phe-L-Pro) isolated from the cell-free supernatant of LAB with antifungal activity, had inhibitory effects on *Ganoderma boninense*, with MICs of 8.2, 8.1, 9.0, 8.4, 6.8 mmol/L, respectively (Kwak et al., 2014). Furthermore, Kwak et al. (2018) isolated 15 cyclic dipeptides containing proline and a single cyclic dipeptide without proline from *Lactiplantibacillus plantarum* LBP-K10, all of which exhibited antifungal activity. Combinations of multiple cyclic dipeptides showed higher antifungal activity than single cyclic dipeptides, with MICs between 18.6 and 22.7 mg/L against *Candida albicans*. Cyclic dipeptides can also inhibit fungal biofilm formation. Li et al. (2022b) isolated cyclo (leu-pro) and cyclo (phe-pro) from the cell-free supernatant of *Lactiplantibacillus plantarum* CCFM8724 and found that these two cyclic dipeptides decreased the expression of virulence genes in *C. albicans* (ALS3 and HWP1 genes), achieving a reduction of biofilm formation. Notably, cyclic dipeptides isolated by Ebrahimi et al. (2020) from the cell-free supernatant of *P. pentosaceus* also reduced aflatoxin content, and exhibited the most antifungal activity, reducing aflatoxin G1 and G2 by 82.06% and 87.32%, respectively.

**Reuterin**

Reuterin is a non-protein, broad-spectrum antimicrobial agent secreted by *Limosilactobacillus reuteri*, which controls growth of Gram-positive and Gram-negative bacteria, and fungi. It is water-soluble, heat-resistant, and highly stable (Cleusix et al., 2007). The mechanism of reuterin's inhibition of fungi may be oxidative stress induced by modification of thiol groups in proteins and small molecules (Schafer et al., 2010). The growth of *Fusarium culmorum*, *Aspergillus niger*, and *Penicillium expansum* spores were inhibited by reuterin produced by *Limosilactobacillus reuteri* R29; the cell-free supernatant with the highest concentration of reuterin completely prevented the growth of all three fungal spores. The MIC<sub>50</sub> of reuterin against *Fusarium culmorum* was 4 mmol/L, while its MIC<sub>50</sub> against both *Aspergillus niger* and *Penicillium expansum* was 8 mmol/L (Schmidt et al., 2018). Reuterin produced by *Limosilactobacillus reuteri* completely inhibited the growth of *Penicillium expansum* at a concentration of 10 mmol/L, and the concentration of reuterin was positively correlated with antifungal effects, within a specific range (Ortiz-Rivera et al., 2017).

**Nucleoside (Cytidine)**

Nucleosides are the basic elements of biological cells that maintain life, participate in the metabolic processes of DNA, and have a variety of anti-tumor, anti-viral, and antifungal functions (Dan et al., 2021). Ryan et al. (2011) isolated and identified two nucleosides with antifungal activity, cytidine and 2’-deoxycytidine, from the cell-free supernatant of *L. amylovorus* LA 19280, and observed that these nucleosides showed antifungal activity against *Aspergillus fumigatus*, with MIC values >200 mg/ml. Chen et al. (2021a) also detected cytidine and 2’-deoxycytidine in the cell-free supernatant of *Lactobacillus kefiri* M4 and *Pediococcus acidilactici* MRS-7, which retarded the growth of *Penicillium expansum*. In addition, nucleosides were also detected in the cell-free supernatant of other types of LAB, such as *Lactiplantibacillus plantarum*, *Propionibacterium freudenreichii*, *Limosilactobacillus reuteri*, and *L. brevis* (Le Lay et al., 2016a; Yépez et al., 2017). However, the studies of cytidine produced by LAB are limited, and cytidine's antifungal mechanisms are still unclear.

**Exopolysaccharides**

Exopolysaccharides (EPSs) are biopolymers produced by LAB, mainly during their growth and metabolism period. EPS are secondary metabolites of microorganisms, can improve the texture and nutritional value of food, and inhibit growth of pathogens and fungi (Moradi et al., 2021). Rodrigues et al. (2005) reported that Kefiran, an edible, biodegradable, and water-soluble EPS produced by LAB, could prevent growth of bacteria and fungi, with MIC of 462 mg/L against *C. albicans*. Nehal et al. (2019) found that EPS produced by *L. lactis* F-MOU also exhibited significant antifungal effects against *C. albicans*, with MIC of 16 mg/ml. EPS inhibition of the growth of pathogenic and spoilage bacteria and fungi may be through the disordering of cell division, destruction of cell membrane and plasma membrane, and decomposition of DNA (Wu et al., 2010). EPS can interact with fungi, depending on their cell membrane permeability, then attack the respiratory chain and cell division machinery, leading to cell death (Abinaya et al., 2018). EPS can also act as a barrier, preventing the input of nutrients to pathogenic bacteria and fungi, thus slowing down their growth; this barrier effect might increase with increased polysaccharide concentration (Han et al., 2016). In addition, EPS could degrade the biofilm of *C. albicans* and reduce the adhesion of other fungi (Abinaya et al., 2018). Similarly, Wang et al. (2020) also found that EPS (composed of glucose, galactose, mannose, and arabinose) produced by *Lactobacillus fermentum* S1 showed activity against biofilms. EPS reduces hydrophobicity, zeta potential, and interactions between cells forming biofilms. The antibiofilm activity of EPS might be related to modifying the bacterial and fungal cell surfaces, inhibiting the attachment of pathogenic and fungal cells to the surface or downregulating the gene expression involved in biofilm formation (Wang et al., 2015).

**FACTORS AFFECTING PRODUCTION OF ANTIFUNGAL COMPOUNDS BY LACTIC ACID BACTERIA**

Different LAB produce different types of antifungal compounds. In addition to the species of LAB, parameters including
temperature, time of incubation, nutritional factors, and growth medium also affect the production of antifungal compounds by LAB (Oliveira et al., 2014).

Crowley et al. (2013b) studied the effect of temperature on the production of antifungal compounds using six antifungal LAB, and they reported that the highest antifungal activity was recorded at 30°C. Valerio (2004) reported the effect of time of incubation on the production of antifungal compounds by Lactiplantibacillus plantarum ITM21B, and it was found that the maximum production of PLA and OH-PLA was reached after 72 h of incubation.

Mu et al. (2009) optimized the medium components for the production of PLA by Lactobacillus sp. SK007. And maximal PLA was obtained when the medium included 30 g/L glucose, 3 g/L K$_3$HPO$_4$, 5 g/L phenylpyruvic acid (PPA), 30 g/L yeast powder, 3 g/L CH$_3$COONa, 47 g/L corn steep liquor, and 3 ml/L Tween-80. Roy et al. (1996) found that compared to M17 and MRS growth media, the cell-free supernatant of L. lactis subsp. lactis CHD-28.3 in Elliker's broth showed optimum antifungal activity against Aspergillus flavus. Magnusson and Schnürrer (2001) reported that PLA yield were increased by the addition of peptides, α-ketoglutarate, citric acid in growth medium. Similar results were also obtained by Schmidt et al. (2018). The production of PLA was significantly increased by the addition of 1.5% phenylalanine (w/v) to MRS broth. And, a maximum yield of reuterin and the highest fungal inhibition were achieved by supplementation of 500 mmol/L glycerol and a reduced glucose content (1.5%) in MRS broth. The synthesis of reuterin was also correlated with temperature, pH, microbial concentration, oxygen concentration, and culture time (Ortiz-Rivera et al., 2017).

**APPLICATION OF ANTIFUNGAL LAB IN BAKERY PRODUCTS**

Sourdough is a natural dough improver that can be used in making bakery products (Nionelli and Rizzello, 2016). Traditionally, the early sourdough (sourdough type I) is obtained after spontaneous fermentation of cereal flour using continuous backslopping. The microorganisms of spontaneous fermentation originate mainly from flours and processing equipment. However, sourdough type I has some disadvantages, including a long fermentation cycle and unstable properties. Therefore, the use of sourdough fermented with certain LAB species together with baker's yeast for dough leavening is more attractive (Drakula et al., 2021). Sourdough fermentation improves the rheological properties of the dough and the nutritional properties of bakery products. This technique also has the potential to inhibit growth of fungi and extend the shelf-life of bakery products.

**LAB Used to Extend the Shelf-Life of Bakery Products**

Sun et al. (2020) added Lactiplantibacillus plantarum LB-1 suspension, which had strong antifungal activity against Aspergillus ochraceus, Aspergillus niger, Fusarium graminearum, Aspergillus flavus, Aspergillus fumigatus, and Penicillium citrinum, into dough, and doubled the shelf-life of whole wheat bread, from 3 to 6 days. Sourdough inoculated with Lactiplantibacillus plantarum CH1 and Leuconostoc mesenteroides L1 showing antifungal activity was added to bread artificially contaminated with Aspergillus tubingensis or Aspergillus flavus, and the shelf-life of bread was extended by 1 day (Ouiddir et al., 2019).

Bartkiene et al. (2018) confirmed that a combination of 15% antifungal Lactiplantibacillus plantarum LUHS135 sourdough, and cranberry coating increased the shelf-life of bread by 6 days, and the shelf-life increased with the amount of sourdough added. However, since excessive sourdough affected the quality of bread, the amount of sourdough added should be determined based on the specific strain selected.

Interestingly, LAB show different antifungal activity in sourdough made with different cereals. Axel et al. (2016) confirmed the above views, and they reported that Limosilactobacillus reuteri R29 and L. brevis R28 with antifungal activity were inoculated into quinoa and white rice flour, for bread making. However, the shelf-life of quinoa bread and white rice flour bread inoculated with the same LAB were different. The concentration of carboxylic acid in quinoa sourdough was much higher than that in rice sourdough, which indicated that the level of metabolites produced by LAB in different cereal sourdough varied. Quattrini et al. (2019) prepared wheat sourdough bread and flaxseed sourdough bread with antifungal LAB, and showed that wheat sourdough fermented with L. hammsesii DSM16381, Lactiplantibacillus plantarum C264, and L. brevis C186 greatly extended the shelf-life of bread contaminated by Aspergillus niger; however, there was no significant effect on bread contaminated with Penicillium roqueforti. Furthermore, they also found that the combination of ricinoleic acid and wheat sourdough greatly extended the shelf-life of bread contaminated with Aspergillus niger or Penicillium roqueforti, compared with linseed sourdough fermented with the same LAB. More examples of using LAB to extend the shelf-life of bakery products can be found in Table 1.

**LAB Used to Improve the Baking Characteristics of Bakery Products**

Antifungal LAB not only prolong the shelf-life of bakery products but also improve the aroma profile and texture of bakery products (Moghaddam et al., 2020). Rizzello et al. (2011) baked using wheat germ sourdough, which not only produced bread with extended shelf-life but also with the lowest hardness and maximum specific volume. L. brevis AM7 with antifungal activity was inoculated into bread hydrolysate, and then the bread hydrolysate was employed for bread making. The specific volume of bread supplemented with 18% bread hydrolysate was improved compared to bread with lower percentages of bread hydrolysate (Nionelli et al., 2020). Bread containing hop extract (25%, vol/ wt) sourdough also had a similar effect, prolonging the shelf-life of bread by 7 days, and enhancing its nutritional value, phytase activity, total phenolic content, and antioxidant activity. In addition, although bread with added hop extract had increased...
bitterness in taste and odor, and reduced sensory score, LAB fermentation overall improved the aroma profile and sensory acceptability of the bread (Nionelli et al., 2018).

The combination of antifungal LAB and fruit fermentation substrate also increased the shelf-life of bakery products and improved their texture, flavor, and nutritional value. The combination of LAB and pitaya inhibited growth of mold; LAB also promoted the release of phenolic acids from pitaya and the conversion of insoluble dietary fiber to soluble dietary fiber. These changes probably favored the formation of covalent and non-covalent linkages with S–S bonds in dough, helping to maintain the integrity of the gluten network structure (Omedi et al., 2021). After fermentation by LAB, the molecular properties of phenolic compounds in fruits can be changed to produce new derivative compounds with more bioactive potential, thereby increasing their antioxidative properties (Massot-Cladera et al., 2014; Septembre-Malaterre et al., 2018). In addition, LAB can use different kinds of fruit sugars to produce different types of EPS. EPS is a natural, high molecular weight polymer with high viscosity, which can be used as a stabilizer and thickener to improve the rheological properties, taste, porosity, and specific volume of bakery products (Li et al., 2022a). A comprehensive list of LAB with potential for improving the baking characteristics of bakery products is listed in Table 2.

### LAB Used to Improve the Safety of Bakery Products

Grains, the raw material of bakery products, are prone to fungal contamination and may be contaminated by mycotoxins

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**TABLE 1** Application of antifungal LAB in prolonging the shelf-life of bakery products.

| Raw material of sourdough | Antifungal LAB | Activity spectrum | Impact on the shelf-life of bakery products | References |
|---------------------------|----------------|------------------|-------------------------------------------|------------|
| Wheat flour               | Lactobacillus sakei KTU05-6, Pediococcus acidilactici KTU05-7, Pediococcus pentosaceae KTU05-8, Pediococcus pentosaceae KTU05-9, Pediococcus pentosaceae KTU05-10 | Penicillium expansum, Aspergillus versicolor, Penicillium chrysogenum, Fusarium culmorum, Aspergillus fumigatus, Candida parapsilosis, Debaryomyces hansenii | Shelf-life extended by 0.5–4.5 days | Cizeikiene et al., 2013 |
| Wheat flour               | Lactiplantibacillus plantarum CRL 778, Lactiplantibacillus plantarum 1A7 | Fungi in the environment | Shelf-life enhanced by 14 days | Gerez et al., 2015 |
| Peas, lentils, and broad bean flour | Lactiplantibacillus plantarum KCCM12515P | Penicillium expansum, Aspergillus niger CH2, Aspergillus japonicas CH6, Penicillium roquefortii CH4, Penicillium digitatum CH10, Metschnikovia pulcherrima CH7 | Shelf-life enhanced by 6 days | Bustos et al., 2018 |
| Whey                      | Lactiplantibacillus plantarum CECT 220, Lactiplantibacillus plantarum CECT 221, Lactiplantibacillus plantarum CECT 223, Lactiplantibacillus plantarum CECT 748 | Penicillium expansum CECT 2278, Penicillium brevicompactum CECT 2316 | Enhanced the shelf-life of bread by 1–3 days | Izzo et al., 2020 |
| Rye and wheat             | Lactiplantibacillus plantarum LUHS135, Leuconostoc mesenteroides LUHS242 | Aspergillus flavus | 2 Days extension in shelf-life | Bartkiene et al., 2018 |

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**TABLE 2** Application of antifungal LAB in improving the baking characteristics of bakery products.

| Antifungal LAB | Impact on the shelf-life of bakery products | Impact on the baking characteristics of bakery products | References |
|----------------|-------------------------------------------|----------------------------------------------------------|------------|
| Pediococcus pentosaceae LUHS183, Pediococcus acidilactici LUHS29, Lactococcus paracasei LUHS244, Lactococcus brevis LUHS173, Lactiplantibacillus plantarum LUHS135, Leuconostoc mesenteroides LUHS242 | Shelf-life extended by 2–7 days | Increased the specific volume of bread and reduced acrylamide content | Bartkiene et al., 2018 |
| Lactiplantibacillus plantarum CECT 220, Lactiplantibacillus plantarum CECT 221, Lactiplantibacillus plantarum CECT 223, Lactiplantibacillus plantarum CECT 748 | 2 Days extension in shelf-life | Increased the sensory score and specific volume of bread and decreased hardness | Karimi et al., 2020 |
| Lactiplantibacillus plantarum FST 1.7, Lactiplantibacillus plantarum L9, Weissella confusa A16 | Shelf-life extended by 3 days | Decreased the hardness of bread and increased elasticity | Moore et al., 2008 |
|                      | 7 Days extension in shelf-life | Maintained global appreciation unchanged | Ran et al., 2022 |
|                      | Shelf-life extended by 1–4 days | Increased the specific volume, sensory score, and moister mouthfeel of bread and decreased hardness | Wang et al., 2022c |
The formation of acrylamide during the growing or storage period, potentially harming consumer health. LAB inhibit growth of fungi and reduce the contents of aflatoxins B1, B2, G1, and G2. LAB-induced degradation of mycotoxins is achieved with binding of the toxin to the bacterial cell wall (Sadeghi et al., 2019). Saladino et al. (2016) reported that Lactiplantibacillus plantarum strains used in fermentation of sourdough reduced bread Aflatoxin B1 levels by 99.9% as compared to the control.

In addition to mycotoxins, acrylamide, which is potentially carcinogenic and neurotoxic to humans, is also a common contaminant of bakery products (Capuano and Fogliano, 2011). Fermentation with selected LAB strains can inhibit the growth of fungi and reduce acrylamide levels in bakery products, which improves the safety of bakery products. The addition of selected LAB, in combination with the antimicrobial cranberry-based coating, resulted in wheat bread with extended shelf-life and reduced acrylamide content. Moreover, the content of acrylamide gradually decreased with increasing amounts of sourdough (Bartkiene et al., 2019). The formation of acrylamide has primarily been related to reducing sugar content (Nguyen et al., 2016). LAB metabolize carbohydrates during fermentation, which reduces the reducing sugar content in dough, in turn leading to the reduction of acrylamide content in dough. In addition, the increase in organic acids also contributes to the reduction of acrylamide content in bakery products (Capuano and Fogliano, 2011).

Furthermore, the use of LAB as a biological preservative greatly reduces the addition of chemical preservatives. The results from Ryan et al. (2008) showed that the combination of sourdough and 0.1% calcium propionate effectively extended the shelf-life of bread, which was similar to bread supplemented with 0.3% calcium propionate alone. Luz et al. (2021) also found that relative to bread supplemented with 0.3% calcium propionate and 3 g/kg lactic acid, bread supplemented with 25 ml Lactiplantibacillus plantarum TR7 fermented whey had its shelf-life extended by 1 day, while the shelf-life of bread supplemented with 50 ml fermented whey was extended by 2 days. Ryan et al. (2011) also similarly found that 0.3% calcium propionate-supplemented bread had weaker antiseptic effects than bread fermented by L. amylovorus DSM 19280. The LAB-fermented bread also greatly reduced the addition of chemical preservatives and improved bakery products’ safety. More examples of the use of LAB to improve the safety of bakery products can be found in Table 3.

**TABLE 3 | Application of antifungal LAB in improving the safety of bakery products.**

| Antifungal LAB | Impact on the shelf-life of bakery products | Impact on the safety of bakery products | References |
|----------------|---------------------------------------------|----------------------------------------|------------|
| Livosilactobacillus reuteri 5529, Lactobacillus spicheri O15, Lactobacillus citreum L123, Lactobacillus amylovorus DSM 19280 | Delayed the growth of fungi in milk bread rolls | Compared with potassium sorbate, sodium benzoate, and calcium propionate, LAB had better preservative effect and reduced the addition of chemical preservatives | Le Lay et al., 2016b |
| Lactobacillus coryniformis LUHS71, Lactobacillus curvatus LUHSV1, Lactobacillus farraginis LUHS206, Leuconostoc mesenteroid LHS225 | Enhanced the shelf-life of bread by 6–7 days | The preservative effect of bread combined with sourdough and low-salt was much better than bread added standard salt (without sourdough) and reduced the addition of salt | Belz et al., 2019 |
| Lactobacillus sanfranciscensis | Shelf-life extended by 3 days | Decreased the acrylamide content of bread | Bartkiene et al., 2019 |
| Livosilactobacillus fermentum IAL 4541, Wickerhamomyces anomalus IAL 4533 | Shelf-life extended by 1–35 days | The preservative effect of bread added 60 g sourdough/100 g dough was similar to bread added with calcium propionate and reduced the addition of chemical preservatives | Debonne et al., 2019 |
| | Shelf-life enhanced by 70 days | Compared with calcium propionate, LAB had better preservative effect and reduced the addition of chemical preservatives | Facco Stefanello et al., 2019 |

**SUMMARY AND PROSPECT**

In conclusion, LAB have extensive application prospects in bakery products. As a new kind of biological preservative, LAB can inhibit the growth of fungi through the production of organic acids, antifungal peptides, hydrogen peroxide, diacetyl, and other antifungal compounds, which greatly extends the shelf-life of bakery products and improves the baking characteristics and safety of products. The inhibitory effects of LAB on fungi are closely linked to the presence of antifungal compounds, with synergistic effects between various compounds. Therefore, LAB capable of producing a variety of antifungal compounds should be screened for use in bakery products. Additionally, we should also pay attention to LAB with antifungal activity and other properties that enhance the flavor profile and texture of bakery products. Lastly, LAB may be incorporated into packaging of bakery products for better protective function.

**AUTHOR CONTRIBUTIONS**

RX: conceptualization and writing—original draft. SZ, YW, XA, QL, and JL: writing—original draft. BH: writing—original draft.
and writing—review and editing. KH, YY, and SL: writing—review and editing. AL: funding acquisition, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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