Chapter 6

Lactic Acid Bacteria in Biopreservation and the Enhancement of the Functional Quality of Bread

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1. Introduction

LAB have a long history in preserving foods from spoilage microorganisms - they are commonly used in food fermentation, may produce several metabolites with beneficial health effects and, thus, are generally recognized as safe (GRAS). The increasing resistance of food spoilage microorganisms to current preservatives, the consumer's high demand for safe, minimally processed foods and the hazards associated with the use of high doses of chemical preservatives has led to the need for finding safer alternatives in food preservation. The application of LAB with the simultaneous control of factors that affect fungal growth can help to minimize food spoilage. The selection and addition of novel isolates of LAB may be the key to reducing the use of chemicals, enhancing nutrients and extend the shelf life of bakery products. In this chapter, the focus will be on the use of LAB as biopreservative agents to extend the shelf life of bakery products and the inhibition of the common spoilage fungi of bread.

2. Sources of LAB

LAB are found in many habitats and occur naturally in a variety of food products, such as dairy, vegetables and meat products (Carr et al., 2002), all of which are rich in the nutrients required for the fastidious metabolism of LAB (Björkroth & Holzapfel, 2003; Hammes & Hertel, 2003). Some LAB are associated with the mouth flora, intestine and vagina of mammals (Whittenbury, 1964), while others are present in fermented seafood, such as *Lactobacillus plantarum* (IFRPD P15) and *L. reuteri* (IFRPD P17), which are reported to be associated with plaa-som fermented Thai fish (Saithong et al., 2010). LAB are the most important bacteria used in the fermentation industry of dairy products, such as yogurt, cheese, sour milk and butter, and in combination with yeast are commonly used to ferment cereal products such as dough (Lavermicocca et al., 2000; Muhialdin et al., 2011a; Ryan et al., 2008).

3. Spoilage fungi in food

The economic losses and the health hazards of the mycotoxins produced by spoilage fungi are the main concerns of the food industry (Gray & Bemiller, 2003). According to Gerez et al., (2009) the spoilage of bakery products by fungi is more common in countries with a high humidity and temperature. Pitt and Hocking (1999) estimated that about 5-10% of food production is spoiled by the growth of yeast and fungi in food materials. Similarly, in Western Europe, the growth of the spoilage fungi of bread is estimated to reach more than 200 million Euros per year (Legan, 1993; Schnürer & Magnusson, 2005). The history conditions of the food can be a major factor in determining any fungal spoilage - for example, stored and processed foods are more sensitive to spoilage when compared with fresh and prepared foods. *Aspergillus* and *Penicillium* species are the most common spoilage fungi for many foods and feeds while *Fusarium* species are reported to attack the cereal grains in the field (Samson et al., 2000).

The most widespread species of fungi that contaminate bakery products belong to the genera *Aspergillus*, *Penicillium*, *Eurotium* (Abellana et al., 1997; Guynot et al., 2005), *Monilia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium* and *Rhizopus* (Lavermicocca et al., 2000, 2003). In addition, fungi may be responsible for off-flavours, the production of mycotoxins and allergenic compounds. There are more than 400 known mycotoxins produced by different fungi (Filtenborg et al., 1996). Mycotoxigenic fungi such as *Aspergillus*, *Fusarium* and *Penicillium* are serious hazards for human health. The six classes of mycotoxins frequently encountered in different food systems are: aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone (Dalié et al., 2009).

4. Common techniques to control spoilage fungi in bakery products

Two types of techniques/factors are commonly used to control spoilage fungi: physical ones such as drying, freeze drying, cold storage, modified atmosphere storage, irradiation, the pasteurization of packaged bread and heat treatment; and chemical ones, in general based on the use of organic acids such as propionic acid and its salts (Farkas, 2001; Legan, 1993). Heat treatment is one of the most important physical factors in controlling fungi growth and mycotoxin production, as mycotoxins are destroyed by heat, although the effectiveness of destruction is affected by the food matrix and the composition of the mycotoxin (Scott, 1984). Mycotoxins have different heat stability - for example, ochratoxin A is highly stable even at 200 ºC (Trivedi et al., 1992), aflatoxins are destroyed only at temperatures of approximately 250 ºC (Levi, 1980), while zearalenone and fumonisin require high temperatures between 150-200 ºC to be efficiently destroyed (Bennett et al., 1980). Microwaves are effective in destroying mycotoxins - the aflatoxin in peanuts is reported to be destroyed using microwaves at a power level of 1.6 kW for 16 min and at 3.2 kW for 5 min (Luter et al., 1982). Among the physical methods, a modified atmosphere and gamma irradiation are preferred to the chemical methods and they have been used successfully in grain storage (Shapira & Paster, 2000).

Chemical methods that use weak acids and salts such as propionic, sorbic and benzoic acids, are usually applied only to inhibit the growth of spoilage microorganisms. The allowable concentrations of sorbate, propionate and ethanol have a limit up to 0.2% (wt/wt), 0.3% (wt/wt) and 2% (wt/wt) respectively. The use of such low concentration may not be sufficient to prevent the growth of spoilage fungi (Dantigny et al., 2005; European Union, 1995). Propionic acid is inhibitory to fungi and *Bacillus* spores and has commonly been used to preserve bakery products. Its activity relies on the un-dissociated form which, at low pH, has optimum activity (Coda et al., 2008; Pattison et al., 2004). The use of propionic acid at a concentration of 4% led to the appearance of cancer-like tumours in rats and eventually led to the prohibition of the use of calcium propionate in some European countries (Pattison et al., 2004). There is a major concern with microorganisms that can develop resistance to chemical preservatives, namely food spoilage and human pathogen fungi resistant to antibiotics and chemicals additives, such as sorbic and benzoic acids (Brul & Coote, 1999; Lourens-Hattingh & Viljoen, 2001). Calcium propionate has been reported to inhibit the growth of many fungi but, after a lag phase, it stimulated the growth of resistant strains of *Penicillium roqueforti* (Suhr & Nielsen, 2004). Interest in natural bio-preservation from LAB has been on the rise as an alternative to chemical preservatives.

5. Significance of the metabolites of LAB

LAB are well known for their antifungal activity, which is related to the production of a variety of compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide, phenyllactic acid, bacteriocins and cycle peptides (Gerez et al., 2009; Lavermicocca et al., 2000; Magnusson et al., 2003; Prema et al., 2008). These compounds were added to several foods in order to conserve them from food-borne and spoilage microorganisms. Organic acids are the main product of LAB in the fermentation systems of the raw materials. The main acids produced by LAB are lactic acid and acetic acid, besides certain other acids depending upon the strain of LAB (El-Ziney, 1998). These acids will be diffused through the membrane of the target organisms in their hydrophobic un-dissociated form and then used to reduce the cytoplasmic pH and stop metabolic activities (Piard & Desmazeaud, 1991). Other factors that contribute to the preservative action of the acids are the sole effect of pH, the extent of the dissociation of the acid and the specific effect of the molecule itself on the microorganisms (Axelsson, 1998).

Bacteriocins exhibit good potential for use in the food industry and as bio-preservation agents (Ennahar et al., 1999). Bacteriocins are small, ribosomally synthesized, antimicrobial peptides or proteins that display inhibition activity toward related species, with no reports about fungal inhibition (Cotter & Ross, 2005). The notable property of LAB supernatant is the heat stability of the antifungal compounds present in it. This will promote the use of LAB supernatant and/or antifungal compounds in heat-treated foods. The supernatant of certain LAB observed to be active within a wide range of pH, starting from as low as 3 and up to 9 depending upon the strain (Muhialdin et al., 2011b). This could be considered as a major factor whereby LAB are used in food preservation when compared with the chemical preservative which are usually active at low pH between 3 and 4.5. Additionally, LAB have a broad spectrum of

antifungal activity against several food spoilage and mycotoxin-producing fungi while commercial preservatives are usually used to control only one or few fungi.

6. Bioactive compounds as antifungal agents

Several lactobacilli species are reported to have antifungal activity (Gerez et al., 2009; Muhialdin et al., 2011b; Plockova et al., 2001; Stiles et al., 1999). The antifungal compounds consist of organic acids, reuterin, hydrogen peroxide and other peptides (Table 1). The organic acids are active at low pH and the activity relies on the un-dissociated form of the acids. Recently, interest has dramatically increased in the use of bioactive peptides produced by LAB as an antifungal agent. The use of protein-like compounds are preferred over the use of acids because their activity is present over a wide range of pH and they are heat stable compounds which are ideal for use in heat processed foods (Muhialdin et al., 2011a). Cyclic dipeptides cyclo (Phe-Pro) and cyclo (Phe-OH-Pro) were produced by the *L. coryniformis* subsp. *coryniformis* Si3 strain and were inhibitory to *Aspergillus* sp. (Magnusson, 2003; Ström et al., 2002). Ryan et al. (2011) observed that sourdough made with *L. amylovorus* DSM 19280 had a longer shelf life compared with bread produced with calcium propionate. The selected strain inhibited the growth of *Fusarium culmorum* FST4.05, *Aspergillus niger* FST4.21, *Penicillium expansum* FST4.22, *Penicillium roqueforti* FST4.11 and *L. amylovorus* DSM 19280 and produced seventeen antifungal compounds.

Compound	Producer	Inhibited fungi	References
Possibly proteinaceous	*Pediococcus acidilactici*	*Saccharomyces cerevisiae*	Vandenbergh & Kanka (1989)
Possibly proteinaceous	*L. lactis* subsp. *Lactis* CHD 28.3	*A. flavus*, *A. parasiticus*, *Fusarium* spp.	Roy et al. (1996)
Caproic acid, propionic acid, butyric acid, valeric acid	*L. sanfranciscencis* CB1	*Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Monilia* spp.	Corsetti et al. (1998)
Benzoic acid, methylhydantoin, mevalonolactone,	*L. plantarum* VTT E78076	*F. avenaceum*	Niku-Paavola et al. (1999)
phenyllactic and 4-hydroxy-phenyllactic acids	*L. plantarum* 21B	Broad spectrum against bakery spoilage fungi	Lavermicocca et al. (200)
3-Phenyllactic acid, cyclo (Phe-OH-Pro), cyclo (Phe-Pro).	*L. plantarum* MiLAB 393	*F. sporotrichioides* and *A. fumigatus*	Ström et al. (2002)
Hydroxy fatty acids, phenyllactic acid, cyclo(Phe-Pro), cyclo(Phe-OH-Pro),	*L. plantarum* MiLAB14	Broad spectrum	Magnusson et al. (2003)

Compound	Producer	Inhibited fungi	References
Possibly cyclic dipeptide	*P. pentosaceus*	*P. expansum*	Rouse et al. (2008)
diacetyl and hydrogen peroxide	*L. fermentum* and *Leuconostoc mesenteroides*	*Rhizopus oryzae, A. niger, A. flavus, Penicillium* sp and *F. oxysporum*	Ogunbanwo et al. (2008)
Acetic acid, phenyllactic acid	*L. reuteri* 1100	*F. graminearum*	Gerez et al. (2009)
(cyclo(Leu–Leu))	*L. plantarum* AF1	*Aspergillus flavus* ATCC 22546	Yang & Chang (2010)
Four peptides and organic acid mixture	*L. plantarum* LB1 and *L. rossiae* LB5	*Penicillium roqueforti* DPPMAF1	Rizzello et al. (2011)
Mixture of peptides	*L. plantarum* 1A7 (S1A7)	Broad spectrum	Coda et al. (2011)
Possibly protein-like	*L. fermentum* Te007, *P. pentosaceus* Te010, *L. pentosus* G004, and *L. paracasi* D5	*A. niger* and *A. oryzae*	Muhialdin et al. (2011a)
nine carboxylic acids, two nucleosides, sodium decanoate and five cyclic dipeptides	*L. amylovorus* DSM 19280	*A. niger* FST 4.21, *A. fumigatus* J9, *F. culmorum* TMW 4.0754 *P. expansum* FST 4.22 and *P. roqueforti* FST 4.11	Ryan et al. (2011)
3-phenyllactic acid and Benzene acetic acid, 2- propenyl ester	*L. plantarum* IMAU10014	*Botrytis cinerea, Glomerella cingulate, Phytophthora drechsleri Tucker, P. citrinum, P. digitatum* and *F. oxysporum*	Wang et al. (2012)

Table 1. Antifungal compounds produced by lactic acid bacteria and their target fungi

7. Method for determining antifungal activity

Rapid, reliable and sensitive methods for the detection of the antifungal activity of LAB becomes essential in the search for new replacements for chemical preservatives with potential industrial applications.

7.1. Dual agar overlay method

This method has been described by several authors (Magnusson & Schnürer, 2001; Ström et al., 2002; Hassan & Bullerman, 2008) and it is accurate and simple for determining the antifungal activity of LAB isolates. The method consists of inoculating the LAB cells in two 2-cm-long lines and/or small circle spots on a MRS agar surface then incubating the plates at 30 °C for 24-48 h in anaerobic jars. The plates are overlaid with 10 ml of malt extract soft agar (2% malt extract, 0.7% agar; Oxoid) containing different concentrations of the spore inoculant of 10^4 and 10^5 spore/ml. The plates are then incubated aerobically at 30 °C for 48-72 h. The inhibition activity is indicated by the clear zones around the bacterial streaks. The scale for measuring the activity can be recorded as follows: -, no activity; +, no fungal growth on 0.1 to 3% of the plate area; ++, no fungal growth on 3 to 8% of the plate area; and+++, no fungal growth on 8% of plate area. Another way to measure the activity is by recording the clear zone diameter around the isolates streak, which refers to the inhibition of the fungi growth. The dual agar overlay method is also a good method for the screening of the antifungal activity of the supernatant of LAB isolates. The supernatant can be mixed with the de Man, Rogosa and Sharpe (MRS) agar or potato dextrose agar (PDA) and poured into Petri dishes followed by a similar step, mentioned previously. The supernatant can be added to the agar before it is autoclaved in order to determine the heat stability of the antifungal compounds present in the supernatants, which is a good indicator of whether the supernatant is used in heat processed foods.

7.2. Agar well diffusion method

The well diffusion method is another approach for determining the antifungal activity of LAB, described as a simple, accurate and flexible method. It is suitable to determine the inhibition activity of LAB supernatant. A fungi numbering 10^4 -10^5 spore/ml are mixed with the selected agar and allowed to solidify. The wells can be made on a variety of agar surfaces - for example, wells are made on potato dextrose agar if the target is a fungi or on a nutrient agar if the target is a bacteria; the wells are made by using a sterilized cork borer with a diameter of 3 or 5 mm. 50 µl of the same agar is added to each well in order to seal the base so as to avoid leakage. The cell-free supernatants are then added to wells in amounts of 30-80 µl and incubated at room temperature for 3-6 h in order to allow the supernatant to be diffused through the agar. The antifungal activity is recorded by measuring the clear zones' diameters around the wells.

7.3. Dry weight of biomass

The reduction of the biomass of the fungi can be a tool for determining the growth inhibition activity of the supernatant. 50 ml of the supernatant is inoculated into a 250 ml flask containing the growth medium for the target fungi and then the suspension of the fungi spores is added at a concentration of 10^5. The fungal mass is harvested on filter paper and dried in an oven at 50 °C for 2 days. The average of the fungal biomass inhibition can be calculated by comparing the weight of treated fungi with the positive control which contains the fungi and the growth medium with no supernatant.

7.4. Micro-titter 96 well plate

The method is simple, inexpensive and practical for determining antibacterial and antifungal activity. The supernatant of LAB is placed into the wells of 190 µl and inoculated with 10 µl of a conidial suspension containing about 10^4-10^5 spore/ml. The plates are then incubated at 25-30 °C. The control is a conidial suspension placed in the wells in equal amounts without the addition of the LAB supernatant. Fungal growth is observed by the naked eye and determined by measuring the optical density at 560-580 nm, starting from 0 h and repeated every 24 h with a spectrophotometer. The result can be obtained by comparing the OD readings of the control with the treated wells. The method is appropriate for evaluating the MIC, heat stability, enzyme activity and effects of pH for the LAB supernatant.

8. Effect of the addition of LAB on bread quality

8.1. Shelf life

Traditionally, chemical preservatives and fungicides are used to inhibit fungal growth but concerns about environmental pollution and consumer health, along with problems of microbial resistance, favour the demand for alternative methods in controlling the growth of fungi (Druvefors et al., 2005). The shelf life of bread has been reported to be extended when certain LAB strains were added to bread formulations (Muhialdin et al., 2011a; Ogunbanwo et al., 2008; Rizzello et al., 2010; Ryan et al., 2011) (Table 2). The use of safe microbes in bread to extend the shelf life of the product is a great research area. Since LAB isolates are safe for use in foods, they are a significant alternative to chemical preservatives. Several researchers in the area of the bakery industry have successfully added LAB to dough and these strains grew well, producing the desired antifungal compounds in the dough.

Various fungi isolated from bakeries were inhibited by *L. plantarum* (LB1) and *L. rossiae* (LB5) isolated from raw wheat germ. Organic acids and peptides synthesized during fermentation were responsible for the antifungal activity; formic acid had the highest inhibition activity (Rizzello et al., 2011). However, the inhibitory compounds characterized were different, depending upon the LAB strains and flour type used. Dal Bello et al., (2007) characterized lactic acid, phenyllactic acid (PLA), cyclic dipeptides cyclo (L-Leu–L-Pro) and cyclo (L-Phe–L-Pro) produced by *L. plantarum* FST 1.7 and found them to inhibit the growth of *Fusarium* spp. in wheat bread. Ryan et al., (2008) reduced the use of calcium propionate from 3000 ppm to 1000 ppm when using sourdough fermented with *L. plantarum* FST 1.7 (LP 1.7) and *L. plantarum* FST 1.9 (LP 1.9), in which the growth of *A. niger*, *F. culmorum* and *P. expansum* was delayed for over six days while the growth of *P. roqueforti* appeared after three days of incubation at 30 °C. *L. plantarum* VTT E-78076. *Pediococcus pentosaceus* VTT E-90390 was reported to inhibit the growth of rope-forming *Bacillus subtilis* and *Bacillus licheniformis* in laboratory conditions and in the bread when the selected strains were inoculated to sourdough and subsequently 20-30 g of the inoculated sourdough was added to 100 g of wheat dough (Katina et al., 2002). Lavermicocca et al. (2000) found that *L.*

Strains	No. of days	Target fungi	Storage temperature °C	Reference
L. plantarum 21B	7	Broad spectrum	20	Lavermicocca et al., (2000)
L. plantarum	12	*Rhizopus oryzae A. niger A. flavus Penicillium sp. F. oxysporum*	27	Ogunbanwo et al., (2008)
L. brevis AM7	21	*P. roqueforti* DPPMAF1	25	Coda et al., (2008)
L. plantarum	10	*A. niger, F. culmorum,* and *P. expansum*	25	Ryan et al., (2008)
L. plantarum CRL 778, *L. reuteri* CRL 1100, and *L. brevis* CRL 772 and CRL 796	8	*Aspergillus, Fusarium,* and *Penicillium*	30	Gerez et al., (2009)
L. plantarum 1A7 (S1A7)	28	*P. roqueforti* DPPMAF1	25	Coda et al., (2011)
L. amylovorus DSM 19280	14	*F. culmorum* FST 4.05, *A. niger* FST4.21, *P. expansum* FST 4.22, *P. roqueforti* FST 4.11	25	Ryan et al., (2011)
L. fermentum Te007, *P. pentosaceus* Te010, *L. pentosus* G004, and *L. paracasi* D5	9-12	*A. niger* and *A. oryzae*	30	Muhialdin et al., (2011)

Table 2. Delay of the appearance of fungal growth on bread with added lactic acid bacteria cells

plantarum 21B inhibited the bread spoilage fungi *Aspergillus, Fusarium, Penicillium* and *Eurotium*; the active compounds were phenyllactic and 4-hydroxyphenyllactic acids. The growth of *Aspergillus niger* appeared after two days in the control sample while *L. plantarum* 21B delayed the growth of the stated fungi for seven days at 20 °C.

8.2. Flavour

Flavour is one of the most valued sensory attributes in bread - volatile and non-volatile compounds produced during the fermentation of dough contribute to bread's flavour. Reports show that the fermentation of dough with LAB can enhance the aroma and flavour

(Ryan et al., 2011; Muhialdin et al., 2011a). The growth of fungi is responsible for the formation of off-flavours and the production of mycotoxins; adding LAB to dough can prevent the growth of fungi and enhance the flavour of bread. The produced compound plays an important role for any technological application to enhance the flavour, such as diacetyl which gives a buttery flavour. Sourness in white bread indicates spoilage in contrast to the sourness of sourdough bread; for this reason, the search for new LAB for application in white bread becomes essential. Finding a new LAB strain that produces less acid and does not drop the pH below 4 will mark a good strategy for resolving such an issue. The addition of *L. paracasi* D5 and *L. fermentum* Te007 in the production of white bread resulted in an improved aroma and a pleasant caramel-like flavour in the baked bread itself (Muhialdin et al., 2011a).

8.3. Quality and acceptability

The quality of bread produced with LAB as a starter culture was reported to improve the texture and the quality of bread by increasing the air cells (Coda et al., 2008; Katina et al., 2002; Lavermicocca et al., 2000). Baker's yeast - also referred to as 'baking yeast' (*Saccharomyces cerevisiae*) - has the ability to ferment different carbohydrates and produce CO_2; the most important factor involving baking yeast in bread manufacturing is to leaven the dough during the bread's preparation. The presence of antimicrobials in the dough is used to inhibit the growth of spoilage microorganisms that can affect the growth of the baker's yeast and delay the fermentation of dough, thereby resulting in economic losses to the bakery industry (Pattison & von Holy, 2001). Baking yeast is a excellent producer of the necessary flavour and aroma compounds from the products of secondary metabolism (Evans 1990).

Pattison & von Holy (2001) found that the presence of propionic salts reduced the baking yeast activity by up to 34.4% in an *in vitro* study carried out using several natural antimicrobials with positive control calcium propionate. In comparison, lactic acid and acetic acid displayed slight effects on the activity reduction of the yeast compared with the positive control. Baking yeast and lactic acid bacteria commonly have live symbiotically in the natural ecosystem of fermenting food and beverages (Kenns et al., 1991). The volume of the dough was increased by adding sourdough containing *L. amylovorus* DSM 19280 when compared with chemical acidification (Ryan et al., 2011). Rizzello et al. (2010) reported the improvement of bread texture properties and the delaying of the staling of the bread because of the anti-staling effect produced by LAB and the synthesis of antifungal compounds. As mentioned previously, *S. cerevisiae* is responsible of leaving the dough and giving the most desirable texture to the bread.

The key role in achieving the optimum growth and activity of the bakery yeast is played by selecting a LAB that does not exhibit inhibition activity against the bakery yeast. Before choosing the LAB to be added to the dough as a co-starter, a simple experiment can be conducted in order to examine the tolerance of the bread yeast to the selected LAB strain. In a test tube mix of 10 ml water, 5 g of white flour, the LAB strain and baking yeast, we

incubate and observe the production of gas at the top of the tube, which is a good indicator of the yeast activity. Ogunbanwo et al. (2008) isolated LAB from retted cassava and studied the effects of lactic acid bacteria as a starter co-culture in combination with *S. cerevisiae* in order to produce cassava-wheat bread. The improvement in the nutritional contents, physical properties and the extension of the shelf life were reported. Bread produced using *L. acidophilus* and *L. brevis* had the highest acceptability on average in relation to the bread produced with other strains of LAB. The use of LAB in bread in terms of improving the quality of wheat bread, bread volume and crumb structure has been reported (Clarke et al., 2002; Zannini et al., 2009).

8.4. Enhancement of a specific nutrient

LAB fermentation in dough has been approved for enhancing the nutritional value and digestibility of bread. Vitamin B, organic acids and the free amino acids produced through the fermentation of LAB can enhance the nutrients' presence in bread. The human body cannot synthesize B-group vitamins and this is why the body needs an external source of the vitamins. Certain LAB has been proven to synthesize B-group vitamins during the fermentation of foods; at the same time, LAB are considered to be the perfect vehicle for delivering the vitamins to the human body.

There are reports about the production of B-group vitamins by LAB isolates. Keuth and Bisping (1993) described the production of Riboflavin (Vitamin B 2) by *Streptococcus* and *Enterococcus* isolated from tempeh (Indonesian fermented food). Folates were observed to be produced by *L. plantarum* in low amounts (Sybesma et al., 2003). Vitamin B 12 (Cobalamin) was also produced by *L. reuteri* as well as the other groups of vitamin B (Santos et al., 2008). LAB enzymatic activity by proteases that take place during dough fermentation will release small peptides and free amino acids, which are considered to be important nutrients that should be present in bread in high quantities (Thiele et al., 2002). Essential amino acids, including lysine, threonine, phenylalanine and valine were reported to be produced by LAB (Gerez et al., 2006). The enzymes produced by LAB including amylases, proteases, phytases and lipases improve the food quality through the hydrolysis of polysaccharides, proteins, phytates and lipids. Anti-nutrients such as phytic acid and tannins can be reduced by LAB fermentation in food, leading to increased sensory properties of the bread (Chelule et al., 2010). The growth of fungi in food materials can cause the synthesis of allergenic spores and hazardous mycotoxins, which will lead to the reduction of the nutritional value of food stuffs. Adding 4% of fermented sourdough to the white wheat flour improved the texture and physical sensation of the bread. Furthermore, it enhanced the free amino acids, protein digestibility, phytase and antioxidant activities (Rizzello et al., 2010).

9. Starter cultures for the bread industry

Lactic acid bacteria were reported as being used as a starter culture or co-culture in the bread industry with success in terms of survivability in dough (Lavermicocca et al., 2000;

Rezzillo et al., 2011). The use of lactic acid bacteria as an antifungal agent or as a starter culture for bakery and processed foods can solve two global issues; firstly, it can extend the shelf life of the food products, which will reduce their cost and the need for low temperatures, secondly, it will satisfy the high demand of modern consumers for high quality food that is free of chemicals. Above all, the product must be safe with an extended shelf life and good sensory properties.

10. Production of LAB cells and inhibitory compounds

10.1. Growth medium

The growth of LAB and the production of antifungal compounds are largely affected by the food matrix itself (Helander, 1997). Most of the studies regarding the antifungal activity of LAB were done using the universal MRS agar. As demonstrated earlier, there are few studies that evaluate the ability of LAB isolates to produce the active compounds in non-defined media as well as few *in situ* studies. The challenge for the food industry is the need for the high production of biomass and the bioactive compounds using an inexpensive fermentation growth medium. A defined medium is all well and necessary for laboratory screening purposes but it is not suitable for heavy industrial plant. The question here is whether the selected LAB can produce the biomass and maintain the antifungal activity. In our laboratory, *L. fermentum* Te007, *Pediococcus pentosaceus* Te010, *L. pentosus* G004 and *L. paracasi* D5 were used to ferment white bread dough and they maintained the antifungal activity, as detected using MRS agar, indicating that these isolates produced the antifungal compounds in the bread dough (Muhialdin et al., 2011a). *Pediococcus pentosaceus* Te010 was further investigated for its ability to grow in formulated media from plant extracts supplemented with the basic growth needs of LAB, such as vitamins, carbohydrates, nitrogen sources and salts. The results indicated that the selected isolate was able to grow in the formulated media and maintain the production of the antifungal activity but, unfortunately, the compounds have not yet been characterized (unpublished data).

10.2. Growth conditions

The growth conditions of any microbe are the key to success during the fermentation process. As for LAB, the generally optimum temperature for growth is 37 °C for 48 h in anaerobic conditions. This is not exactly what can be applied for the production of antagonistic fungal inhibitor compounds. Some of the LAB are psychrophilic and prefer low temperatures for their growth while others are thermophilic and prefer high temperatures for their growth. This should be considered as a significant factor because the optimum growth temperature has a significant impact on the production of antifungal compounds. As well as temperature, the incubation time has a significant effect on the production of antifungal compounds with respect to the availability of nutrients in the growth medium and the production of primary or secondary metabolites.

11. Future research

The high demand by consumers for foods free of chemical preservatives has led to increasing amounts of research to provide alternatives for these chemicals. LAB provides technologically practicable alternatives for the replacement of chemical preservatives. The achievement of selecting LAB as starter cultures or co-cultures in fermentation processes can improve the desired properties of bread, at the same time providing consumers with new chemical-free foods. There is a need to study the interaction between the food matrix and the kinetics of the starter culture of LAB in bread; such studies will contribute to the bread industry by increasing the yield of the antifungal and nutritional compounds produced by LAB. Besides using the LAB cells in bread formulations, the use of the supernatant of LAB should be considered, especially the supernatant of LAB that are grown in non-conventional media such as plant extract and other cheap materials. Additional studies on the contribution of bioactive molecules to the quality and shelf life of foods will surely widen the use of LAB strains as a novel bio-control strategy in bakery products.

12. Conclusion

LAB can be used as a starter culture or a co-culture in the bread industry to enhance the sensory properties of bread and extend the shelf life. The nutritional value of the bread is enhanced due to the production of free amino acids, organic acids and a variety of Group-B vitamins. The antifungal compounds produced by LAB are important for the food industry for replacing or reducing the use of chemical preservatives. Several methods have been developed to determine the antifungal activity of the cells and the free cell supernatant. Natural sources of food preservatives - especially LAB - are important and reflect one possibility for fulfilling the needs of modern consumers of bakery products that are free of chemicals. Challenges are evident in finding new and novel isolates of LAB that can be applied in bread and which do not affect the activity of the yeast or inhibit their growth. Future works should consider the use of the LAB supernatant as well as the cells because the active compounds can be present in the supernatant. Inexpensive media are also important for high-scale industry, especially the use of plant extracts that are rich in carbohydrates and which can be supplied in bulk over the course of the year.

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