BIOPRESERVATION OF FOOD BY LACTIC ACID BACTERIA AGAINST SPOILAGE FUNGI

Belal J. Muhialdin[1], Zaiton Hassan[1], Sajaa Kh. Sadon[2]
[1]University Sains Islam Malaysia (USIM), Faculty of Science and Technology,
Bandar Baru Nilai, 71800 Nilai Negeri Sembilan, Malaysia
[2] University Tenaga Nasional (UNITEN), Collage of Engineering,
Jalan Ikram-Uniten, 43000 Kajang, Selangor, Malaysia
E-mail: belaljamal.mahialdin@yahoo.com

Abstract
Food industry use chemical preservatives such as propionic, sorbic and benzoic acid to extend the shelf life of food. Interest in natural bio-preservation from lactic acid bacteria has been on the increase as an alternative to be used instead of these chemicals. The use of lactic acid bacteria has become extensively recognized as a natural source of preservatives that improve the quality sensory of processed food and to promote health of the consumers. LAB produce a variety of low molecular weight compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and other metabolites. Many of these metabolites have a broad spectrum against bacteria, fungi and yeasts. Recently, antifungal activity of lactic acid bacteria is introduced to replace the chemical preservatives or to reduce the use of them. The spoilage fungi, food borne bacteria and yeast cause serious problems in stored food; they have bad impact on the economy of the world. Most of these fungi produce mycotoxins such as aflatoxins, trichothecenes, fumonisin, ochratoxin A and patulin. These mycotoxins can affect the health of human. The development of suitable technology to produce and identify the antifungal compounds is required for industrial production. Production of antifungal compounds should be based on the ability to stand high temperature, stress during processing and storage of products. This review summarizes the antifungal activity and the potential applications with the new technologies for selection and identification of LAB isolates.

Keywords: antifungal activity, LAB, organic acid, bacteriocin, biopreservation

Submitted: 27.02.2011 Reviewed: 15.03.2011 Accepted: 25.03.2011

1. INTRODUCTION

Lactic acid bacteria (LAB) are Gram-positive microorganisms, prefer anaerobic conditions but are aero-tolerant, catalase negative, cocci or rods. These groups of bacteria are nonpathogenic, produce organic acids during the fermentation of carbohydrates. They are used in the production of foods prepared by lactic fermentation such as dairy products, fermented fruits, vegetables, and fermented meats (Hammes and Hertel, 2003; Hammes and Tichaczek, 1994; Asmahan, 2010).
LABs are divided into two groups depending on their fermentation pathway: group one, homofermentative bacteria such as *Lactococcus* and *Streptococcus* yield two lactates from one glucose molecule. Group two, heterofermentative such as *Leuconostoc* and *Weissella* produce one lactate, ethanol and carbon dioxide out of one glucose molecule. (Caplice and Fitzgerald, 1999; Kuipers et al., 2000). In addition, LAB produces small organic compounds that give the aroma and flavor to the fermented product (Caplice and Fitzgerald, 1999). LAB contribute to enrichment of the human dietary through development of a wide diversity of flavors, aromas and textures in food through the fermentation process and, enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids and vitamin.

1.1. Antagonistic activity of lactic acid bacteria

Lactic acid bacteria has a long history of use as bio-preservatives for food and feed storage; they are known to produce different antimicrobial compounds that are able to control pathogenic, spoilage bacteria, undesirable spoilage yeast and spoilage fungi

(Dalie et al., 2009, Messens, 2002; Lindgren and Dobrogosz, 1990). Their preserving effect mainly relates to production of organic acid such as lactic acid and acetic acid, hydrogen peroxide, competition for nutrients, production of bacteriocins and protein, or proteinaceous compounds (Stiles, 1996; Lindgren and Dobrogosz, 1990, Ström et al., 2002, Dalie et al., 2009). In addition to the antimicrobial compounds produced by LAB other compounds such as fatty acids (Corsetti et al., 1998), phenyllactic acid (Lavermicocca et al., 2000), peptide, phenyllactic acid, cyclo(Phe-Pro), cyclo(Phe-OH-Pro) and reuterin (Magnusson, 2003), and organic acids, hydrogen peroxide and diacethyl (Messens and De Vuyst, 2002) were reported to have antifungal activity. The compounds that produced by the LAB are:

a. Organic acids

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 beside some other acids depending on the strain of LAB (El-Ziney, 1998). The mechanism of how these acids work as antimicrobial is described by (Piard and Desmazeaud, 1991). The acids diffuse through the membrane of the target organisms in their hydrophobic un-dissociated form and then reduce the cytoplasmic pH and stop metabolic activities. Other factors that contribute to the preservative action of the acids are the sole effect of pH, the extent of dissociation of the acid and the specific effect of the molecule itself on the microorganisms (Axelsson, 1998; Piard and Desmazeaud, 1991).

Food borne pathogens, especially anaerobes and facultative anaerobes, even when they are present in low numbers can grow during storage at low temperatures as in the refrigerators. Some food borne pathogens can multiply in such conditions and posed risk to the safety of raw, processed and stored foods. Many of these microorganisms are Gram-negative bacteria, including *Yersinia enterocolitica* and *Aeromonas hydrophila*; and some of mesophilic species like *Salmonella* spp, pathogenic *Escherichia coli*, Gram positive *Listeria monocytogenes* that are capable of becoming virulent during low temperature storage. The inhibition activity of LAB to the growth of pathogenic bacteria is most likely due to the production of organic acids and bacteriocin (De Vos, 1993; Klaenhammer, 1993).

Corsetti et al., (1998) observed that *Fusarium*, *Penicillium*, *Aspergillus* and *Monilia* were inhibited by mixture of acetic, caproic, formic, propionic, butyric and n-valeric acids. These compounds were detected from obligate hetero-fermentative *Lactobacillus* spp and *L. sanfrancisco* CB1 had the largest antifungal spectrum.

b.Bacteriocin

Several Gram positive and Gram negative bacteria produce different types of bacteriocins (Riley and Wertz, 2002). Bacteriocin has been found in several species of bacteria, but most of bacteriocins studied are from LAB because of their generally recognized as safe (GRAS) status. Bacteriocins that are produced by LAB have good potential to be used in the food industry and as bio-preservation agents (Ennahar et al., 1999; Vandenbergh, 1993). Bacteriocins produced by LAB are small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocins (Klaenhamme, 1988; Cotter and Ross, 2005).

The first report about bacteriocin was made by Rogers (1928) who showed the antagonistic activity for *Lactococcus lactis* against *L. bulgaricus*. The substance was determined to be a polypeptide and named nisin. Its antibacterial spectrum includes inhibition of *streptococci*, *staphylococci*, *Bacillus* spp, *clostridia* and *lactobacilli*. Nisin was permitted for the use as preservative in the UK In 1969, the Food and Drug Administration and World Health Organization permitted the use of nisin as a food ingredient for the dairy and cheese industry (De Vuyst and Vandamme, 1994; Vandenbergh, 1993).

In general, bacteriocins are cationic peptides that display hydrophobic or amphiphilic properties and the bacterial membrane is in most cases the target for their activity. Depending on the producer organism and classification criteria, bacteriocins can be classified into several groups (McAuliffe et al., 2001).

Klaenhammer (1988) divided bacteriocins into three Classes namely,

1) Class I: Lantibiotics or small, heat-stable, lanthionine- containing, single- and two-peptide bacteriocins whose inactive prepeptides are subject to extensive post-translational modification.

2) Class II: Peptide bacteriocins or small, heat-stable, non-lanthionine-containing bacteriocins, including pediocin-like or *Listeria*- active bacteriocins (Class IIa), two-peptide bacteriocins (Cllass IIb), and circular bacteriocins (Cllass IIc).

3) Class III: Bacteriolysins or large, heat-labile, lytic proteins, often murein hydrolases.

However, there are limitations in the use of bacteriocins as bio-preservatives. (Hanlin et al., 1993; José et al., 2007) in that:

1) Some bacteriocins ineffective against spoilage and pathogenic Gram negative bacteria, yeasts and fungi.

2) Their host range can be rather narrow and their effects are limited to certain strains or species, and

3) There are insensitive variants within Gram positive species that appear rather frequently, even in the presence of bacteriocins.

Bacteriocin-like inhibitory substances were also reported to maintain the inhibition activity against non-pathogenic and pathogenic food-associated and human pathogenic bacteria (Corsetti et al., 2005).

The properties of some well characterized bacteriocins produced by different strains of lactic acid bacteria are shown in (Table 1).

Lactobacillus pentosus TV35b produced a bacteriocin-like peptide (pentocin TV35b) that have inhibitory activity against the growth of *Clostridium sporogenes*, *Cl. tyrobutyricum*, *Lact. curvatus*, *Lact. fermentum*, *Lact. sake*, *Listeria innocua*, *Propionibacterium acidipropionici*, *Propionibacterium* sp. and *Candida albicans* (Okkers *et al*., 1999).

Table 1: Bacteriocins produced by different organisms and their properties

acteriocins	Organisme	Properties
Nisin	*Lactococcus lactis* subsp.*lactis* ATCC 11454	Lantibiotic, broad spectrum, chromosome plasmid mediated, bactericidal, produced late in the growth cycle. Broad spectrum, plasmid mediated
Pediocin A	*Pediococcus pentosaceus* FBB61 and L-7230	Broad spectrum
Pediocin AcH	*Pediococus acidilactici* H	Broad spectrum
Leucocin	*Leuconostoc gelidum* UAL 187	Broad spectrum, plasmid Mediated, bacteriostatic, produced early in the growth cycle
Helveticin J Carnobacteriocn	*L. helveticus* 481 *Carnobacterium piscicola* LV17	Narrow spectrum, chromosomally mediated, bactericidal Narrow spectrum, plasmid mediated.

Source: Soomro *et al*., 2002

Moreno *et al*. (2002) reported that LAB isolated from fermented food tempeh produced 3.4 kDa for B1 bacteriocin, and 3.4 kDa and 5.8 kDa for B2 bacteriocins that inhibited the growth of Gram-positive bacteria including *Listeria monocytogenes*. Recently, LAB isolates from Malaysian fermented fish, budu, (*L. casei* LA17, *L. plantarum* LA22 and *L. paracasei* LA02) inhibited the growth of *B. cereus*, *S. aureus*, *Salmonella enterica*, *Listeria*

monocytogenes, E. coli and *Lactococcus lactis* (Liasi *et al*., 2009).

c. Carbon dioxide

Carbon dioxide CO_2 is one of products produced by hetero-fermenters LAB. The activity of CO_2 is due to two factors firstly, it creates anaerobic condition and replaces the existent molecular oxygen in the products and secondly, CO_2 has antimicrobial activity and this activity is important in the vegetable fermentation to prevent the growth of spoilage fungi (Lindgren and Dobrogosz, 1990).

Common fruit spoilage organisms such as *Botrytis, Rhizopus* and *Penicillum* are not inhibited by 10% CO_2 but concentrations between 20 - 50% have strong antifungal activity (Clark and Taklcs, 1980; Bliekstad et al., 1981).

d. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is produced by most of the LAB when oxygen is available (Kandler, 1983). The activity of hydrogen peroxide has been examined in different experiments such as, agar diffusion and broth dilution.

Reports from Venturini et al. (2002) showed that growth of *P. expansum* was completely inhibited by a 5% hydrogen peroxide solution when tested by an agar diffusion assay.

These researchers suggested that the application of small quantities of hydrogen peroxide to apple skin might be an alternative to fungicides to inhibit *P. expansum*. Ponts (2006) reported that the rate of the spore germination of *F. graminearum* may be affected by hydrogen peroxide.

Hydrogen peroxide is well studied and the modes of action are well known. It is related to the strong oxidizing effect on the bacterial cell, and to the destruction of basic molecular structures of cellular proteins (Condon, 1987; Magnusson, 2003).

The inhibition of foodborne pathogens by LAB has been ascribed at least in part to the activity of H2O2 (Gilliland and Speck, 1977).

H_2O_2 produced by *L. lactis* was able to inhibit the growth of *Staphy*lococcus *aureus* at low concentration of 5 µg per ml in broth system. The MRS agar is not recommended for the screening of antimicrobial activity from hydrogen peroxides, the H_2O_2 degraded in the MRS most likely due to catalase activity of the yeast extract. (Rodríguez et al., 1997).

e. Reuterin

Reuterin (3-HPA) is a product produced by some strains of lactic acid bacteria (LAB) from the fermentation of the glycerol.

The production of reuterin was reported from *L. reuteri* (Chung et al., 1989; Axelsson, 1989). Reuterin is active against many kinds of microorganisms including Gram-positive and Gram-negative bacteria, yeast and fungi. Antifungal activity was shown against species of *Candida, Torulopsis, Saccharomyces, Aspergillus* and *Fusarium* (Chung et al., 1989). Other reports also show the production of reuterin from different LAB isolates such as *L. brevis, L. buchneri* (Schütz and Radler, 1984), *L. collinoides* (Claisse and Lonvaud-Funel, 2000). The addition of glycerol to some of the reuterin producing LAB isolates has increased their antifungal activity (Magnusson, 2003). Sobolov and Smiley (1960) suggested that the mode of action of glycerol breakdown by LAB is through dehydration of glycerol to 3-HPA, then oxidized to 3-hydroxypropionic acid or reduced to 1,3-propanediol.

They proposed a mechanism for breakdown of glycerol by lactobacilli by dehydration of glycerol to 3-HPA that further might be oxidized to 3-hydroxypropionic acid or reduced to 1,3-propandiol (Sobolov and Smiley, 1960). Slininger et al. (1983) described the metabolic pathway for the production and further reduction or oxidation of 3-HPA (Figure 1). The glycerol dehydratase of *L. reuteri* has been purified and characterized (Talarico and Dobrogosz, 1990).

The production of reuterin was reported from *L. brevis, L. buchneri, L. collinoides* and *L. coryniformis* (Schütz and Radler, 1984; Claisse and Lonvaud-Funel, 2000; Nakanishi et al., 2002).

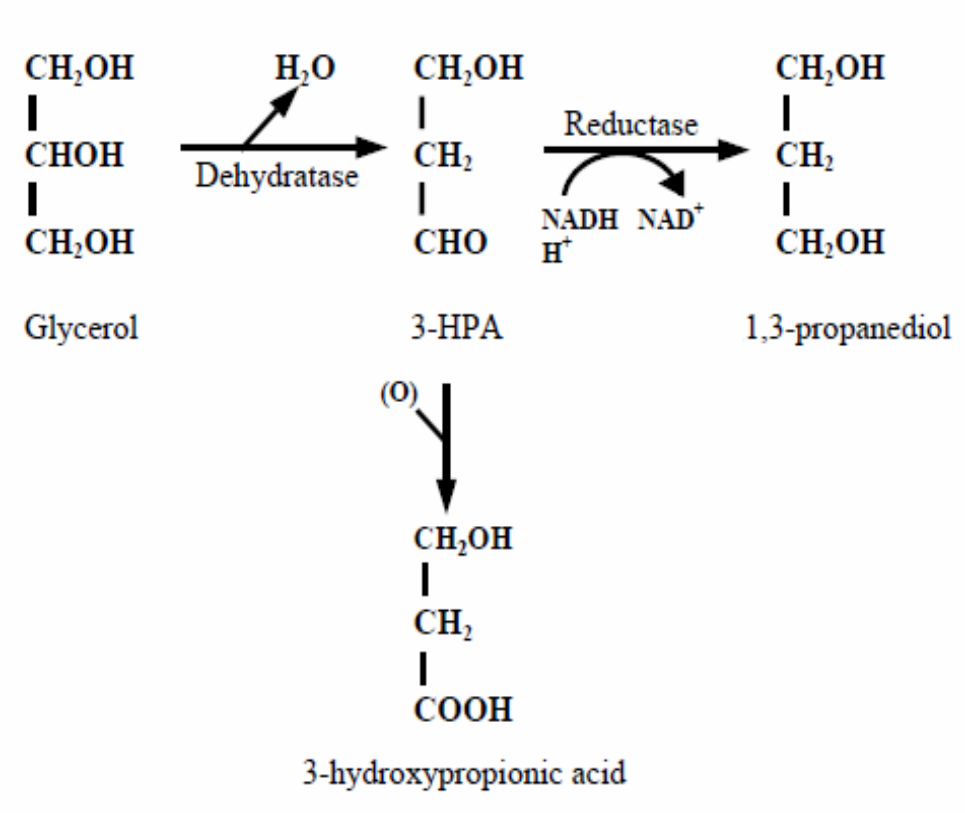

Figure 1: Metabolic pathway for the production and further reduction or oxidation of 3-HPA from glycerol (Slininger *et al*., 1983)

f. Phenyllactic acid

Phenyllactic acid is special organic acid that produced by certain LAB and shows antifungal activity (Gerez et al., 2009). Lavermicocca et al. (2000), Magnusson (2003), Magnusson et al. (2003) and Ström *et al*. (2002), all reported that phenyllactic acid isolated from LAB had spectrum range to inhibit pathogen bacteria, spoilage bacteria and spoilage fungi. Prema et al. (2008) Observed *Lactobacillus plantarum* strain were isolated from grass silage have produced a broad spectrum of antifungal compound. Include 3-phenyllactic acid active against food and feed-borne filamentous fungi in agar plate assay. Sourdough bread started with *L. plantarum* 21B that produce phenyllactic acid have inhibited the growth o the fungi *Aspergillus niger* for 7 days, compared to bread started with a *Lactobacillus brevis* that did not produce phenyllactic acid (Lavermicocca et al., 2000).

1.2. Antifungal activity of LAB

Several reports about the antifngal activity of LAB e.g. *L. casei* (Gourama, 1997), *L. coryniformis* subsp. *coryniformis* (Magnusson and Schnurer, 2001), *L. pentosus* (Okkers et al., 1999), *L. lactis* subsp. *lactis* (Roy et al., 1996) and *L. plantarum* (Niku- Paavola et al., 1999; Ström et al., 2002; Lavermicocca et al., 2003). Suzuki et al. (1991) reported activity of *Leuconostoc mesenteroides* sp. That used in cheese to have antimould activity but the compound was not isolated and identified. Earlier report from Batish et al., (1989) Observed activity of *S. lactis* subsp. *diacetilactis* DRC1 that have inhibition activity against spectrum range of fungi and suggest the activity to be possibly due to proteinaceous compounds. LAB produces several compounds that have antifungal activity (Table 2).

Table 2: Antifungal compounds produced by lactic acid bacteria and their spectrum range of inhibitory activity

LAB	Compounds	Antifungal activity	References
Lactobacillus casei ATCC 393	ND	*Aspergillus parasiticus*	El-Gendy and Marth (1981)
L. casei var rhamnosus	< 1kDa	Broad spectrum	Vandenbergh & King (1988)
Lactobacillus reuteri	3-HPA reuterin	>50 fungi Broad spectrum	Talarico and Dobrogosz (1990)
Pediococcus acidilactici	Possibly proteinaceous	*Saccharomyces cerevesiae*	Vandenbergh and Kanka (1989)
Lactococcus lactis	ND	*Aspergillus parasiticus*	Luchese and Harrigan(1990)
L. casei subsp. *Rhamnosus* LC-705	ND	*Candida lusitaniae*, *Aspergillus* niger, *Fusarium* spp, *Penicillium* spp, *Cladosporium* spp	Mäyrä-Mäkinen et al., (1994)
L. lactis subsp. *lactis* CHD 28.3	Possibly proteinaceous	*Aspergillus flavus*, *A. parasiticus*, *Fusarium* spp	Roy et al., (1996)
L. sanfrancisencis CB1	Caproic acid, propionic acid, butyric acid, valeric acid	*Fusarium* spp, *Penicillium* spp, *Aspergillus* spp, *Monilia* spp	Corsetti et al., (1998)
L. plantarum VTT E78076	Benzoic acid, methylhydantoin, mevalonolactone, cyclo(Gly-L-Leu),	*Fusarium avenaceum*	Niku-Paavola et al., (1999)
L. pentosus	Pentocin TV35b	*Candida albicans*	Okkers et al., (1999)
L. plantarum	Phenyllactic acid, 4-hydroxyphenyllactic acid	Broad spectrum	Lavermicocca (2000)
L. plantarum MiLAB 393	3-Phenyllactic acid, cyclo(Phe-OH-Pro), cyclo(Phe-Pro).	*Fusarium sporotrichioides* and *Aspergillus fumigatus*	Ström et al., (2002)
L. plantarum MiLAB14	Hydroxy fatty acids, phenyllactic acid, cyclo(Phe-Pro), cyclo(Phe-OH-Pro),	Broad spectrum	Magnusson et al., (2003)
P. acidilactici LAB 5	Phenolic compound	*A. fumigatus*, *A. parasiticus*, *F. oxyporum*, *Penicillium* spp	Mandal et al., (2007)
P. pentosaceous	Possibly cyclic dipeptide	*P. expansum*	Rouse et al., (2008)
L. reuteri 1100	Acetic acid, phenyllactic acid	*F. gramminearum*	Gerez et al., (2009)

Source: Magnusson, 2003

1.3. Antifungal protienaceous compounds produced by LAB

Several lactobacillus species produce compounds that antifungal properties (Stiles et al., 1999; Plockova et al., 1999, 2001). *L. rhamnosus* VT1 exhibited strong antifungal properties and capable of inhibiting the growth of many spoilage and toxigenic fungi including species in the genera *Aspergillus, Penicillium* and *Fusarium* (Stiles et al., 1999). Bullerman et al. (2006) also reported that *Lb. paracasei* ssp. *tolerans* completely inhibited the growth of *Fusarium proliferatum* M 5689, M 5991 and *F. graminearum* R 4053 compared to controls by dual agar plate assay. A novel proteinaecous compound was isolated from *Lb. plantarum* VTT E78076 that was inhibitory against *Fusarium avenaceum* (Niku-Paavola et al., 1999). Okkers et al., (1999) purified and characterized a peptide TV35b from *L. pentosus* with antifungal effect against *Candida albicans.*

Other kinds of antifungal dipeptides which are cyclic namely, cyclo (Phe-Pro) and cyclo (Phe-OH-Pro) were produced by *L. coryniformis* subsp. *coryniformis* Si3 strain and were inhibitory to *Aspergillus* sp. (Magnusson, 2003; Ström et al., 2002). The peptides are highly heat stable with an estimated molecular weight 3 KDa (Magnusson and Schnürer, 2001). Other LABs also produced antifungal compounds. Roy *et al*. (1996) isolated a *Lactococcus lactis* subsp. *lactis* with inhibition activity against several filamentous fungi. The isolates lose their activity when treated with different enzymes. Different LAB isolate (Weissella *confusa, W. cibaria, Leuconostoc citreum, L. mesenteroides, Lactococcus lactis, L. rossiae* and *L. plantarum*) inhibited the growth of fungi *Aspergillus niger, Penicillium roqueforti* and *Endomyces fibuliger* in sourdough system and potential to use them as natural preservatives (Lavermicocca et al., 2003).
Recently, Rouse *et al*. (2008), observed that *Pediococcus pentosaceus* produced compounds that were inhibitory to *P. expansum. Pc. acidilactici* LAB 5 and *Lb. reuteri* 1100 were reported to inhibit range the growth of spoilage fungi in different food materials (Mandal et al., 2007; Rouse et al., 2008). *Pc. acidilactici* LAB 5 and *Pc. Pentosaceous* were able to extend the shelf life of some bakery products (Mandal et al., 2007; Gerez et al., 2009). *Lactobacillus casei* was reported able to inhibit the growth and the aflatoxins production by *Aspergillus parasiticus* (El-Gendy and Marth, 1981). The supernatant of a mixture of *Lactobacillus* spp isolated from commercial silage able to reduce both mould growth and aflatoxin production by *Aspergillus flavus* subsp. *parasiticus* (Gourama and Bullerman 1995).

1.4. Mechanisms of the antimicrobial activity of compounds produced by LAB

The mechanisms of action of different antimicrobial compounds produced by LAB are not fully understood. The effect of organic acids on inhibitory activity against microorganism is related to the pH value. In dilute solutions, pH is directly related to the concentration of hydrogen ions. The release of hydrogen ions from an acid is dependent on the strength of the acid. Acids produced from fermenting bacteria are weak organic acids (acetic, lactic and propionic acid) (Helena, 2010). These types of acids only partly release their hydrogen ions in the pH range of foods. The plasma membrane of most microorganisms restricts penetration by charged molecules. However, un-dissociated molecules can easily diffuse (Stratford, 1999). Different microorganisms have different respond to the weak acid. The action of lactic acid, and other organic acids, is also believed to act through some other mechanism, since no connection between decrease in internal pH and degree of lactic acid inhibition has been observed (Freese et al., 1973).
The mode of action of bacteriocins produced by LAB is the formation of holes in the membrane of the target organisms, causing leakage and destruction of the trans-membrane potential (Todorov, 2009; Moll et al., 1996). A novel plasmid with antimicrobial activity

protein Dysgalacticin produced by *Streptococcus* subsp. *equisimilis* also disturbs the membrane and inhibits glucose uptake (Swe et al., 2009). Most of the mechanisms of bacteriocins are related to forming of pore in the membranes of the target microorganisms which cause the leakage of the cytoplasm (Jack et al., 1995; José et al., 2007). Similarly, bacteriocins produced from *Escherichia coli* with molecular weight of 40 to 80 was found to cause nuclease/pore-forming which lead to the leakage of nuclease content causing the death of organism (Margaret and John, 2002).

1.5. Bio-preservation of food by LAB

Fungi in foods are potential danger for public health and causing major economic loses. Spoilage of food by fungi have been a global issue and great loss in the economy for the stored, prepared food and feed system. This is especially prevalent in the tropical countries because of the high ambient temperature and high moisture which are suitable conditions for the growth of the fungi. It is the major factor that decreases the shelf life of many foods. Filamentous fungi are common spoilage organisms of food products, such as milk, cheese, fermented meat products, bread, as well as stored crops (Bullerman, 1977; Filtenborg, et al., 1996). The most widespread species of fungi that contaminate bakery products belong to the genera *Eurotium*, *Aspergillus*, *Penicillium* (Abellana et al., 1997; Guynot et al., 2005), *Monilia*, *Mucor*, *Endomyces*, *Cladosporium*, *Fusarium*, and *Rhizopus* (Lavermicocca et al., 2000; Lavermicocca et al., 2003).
In addition, fungi may be responsible for off-flavors and synthesize mycotoxins and allergenic compounds. There more than 400 mycotoxins are well known produced from different fungi (Filtenborg et al., 1996). Mycotoxinogenic fungi such as *Aspergillus*, *Fusarium* and *Penicillium* are serious hazards for the human health. Six classes of mycotoxins frequently encountered in different food systems are: aflatoxins, fumonisins, ochratoxins, patulin, trichothecenes and zearalenone (Dalie et al., 2009). Many techniques are used for the preservation of food and feed system to reduce the fungi growth and spoilage. Physical techniques such as drying, freeze drying, cold storage, modified atmosphere storage, and heat treatment are used for food preservation (Farkas, 2001). In addition, several chemical preservatives have been found to function as food preservatives against yeast and fungi. The organic acids like acetic, lactic, benzoic and sorbic acid are found to inhibit the growth of both bacteria and fungi (Sofos and Busta, 1981; Blocher & Busta, 1983). Propionic acid is inhibitory to fungi and *Bacillus* spores therefor, it is largely used for bread preservation (Pattison et al., 2004).
Suhr and Nielsen (2004) reported that high calcium propionate concentration 0.3% (w/v) has strong inhibitory effect to many fungi, but after the lag phase of growth, it also stimulated resistant strains of *Penicillium roqueforti*. The appearance of cancer in rats fed propionic acid at concentrations of up to 4% has led to the prohibition on the use of calcium propionate in some European countries for bakery products (Pattison et al., 2004). The European instruction on preservatives is to decrease the percentage of sorbate (0.2%, wt/wt) and propionate (0.3%, wt/wt). Food and Agriculture Organization (FAO) recommended the use of ethanol in baked products is permitted to only 2% and below (Dantigny *et al*., 2005).

1.6. Identification methods of LAB

Traditionally, the identification of LAB is by biochemical methods that has been well studied (Ahmed and Kanwal, 2004; Parvathy and Puthuvallil, 2005; Isabel et al., 2000). API 50 CHL kit (API system, BioMérieux, France) is widely used for identification of lactic acid bacteria. This method is simple, and involves the fermentation of different carbohydrates after 48 h of incubation (Conter et al., 2005). However, API test has problems such as the distinguishing between the changes of color after the fermentation of the carbohydrate in the wells. Other issue is the number of the cell

of LAB could be low and not sufficient to ferment the carbohydrate in each well.
Recently, genetic tools and techniques have been developed to identify LAB especially polymerase chain reaction (PCR) based methods such as 16S rDNA, 16S ribosomal RNA (rRNA) 16S rRNA gene sequencing has become prevalent microbiology and biotechnology as a rapid, bacterial identification accurate methods alternative to phenotypic, fingerprinting and restriction fragment length polymorphism (RFLP) in this method the restriction enzymes broke the DNA sample into pieces (digested) and the resulting restriction fragments are separated according to their lengths by gel electrophoresis, pulse-field gel electrophoresis (PFGE) can be used in genotyping or genetic fingerprinting in epidemiological studies of pathogenic organisms, denaturing gradient gel electrophoresis (DGGE), this method can overcome the limitation of conventional separation by agarose gel electrophoresis that results single DNA band and temperature gradient gel electrophoresis (TGGE) are useful method to study nucleic acids like DNA and RNA, as well as proteins. (Holzapfel et al., 2001; Schleifer and Ludwig, 1995; Zoetendal et al., 2002; Shea 2004; Fischer and Lerman, 1980).

1.7. Future applications and potential outcome

Lactic acid bacteria (LAB) have many potential applications in many industrial fields. In food, LAB strains may play in the food industry because of their inhibition activity against spoilage yeast and filamentous fungi therefore, they can be use as starter cultures, co-cultures, or bioprotective cultures, to improve food quality, shelf life and safety. On the other hand, antimicrobial compounds that produced by probiotic LAB may play an important role during in vivo interactions occurring in the human gastrointestinal tract, in addition, contributing to gut health. Lactic acid bacteria (LAB) have a long history of application in the preparation of fermented foods because of their beneficial influence on nutritional and the shelf life characteristics (Leroy and De Vuyst, 2004). LAB antimicrobial compounds also can be used in the medicine industry because of their spectrum activity against Gram positive and Gram negative bacteria since they produce bacteriocins which can be alternative to the known antibiotic. These substances will be promising to inhibit the human pathogen bacteria especially, the pathogens that highly resist the known antibiotic. The indoor applications are other field to use LAB substrates to inhibit the moulds growth, the indoor moulds damages have been increased in the recent years, there are concerns about the moulds growth on the woods and their great loses especially, in the tropic countries when the environment are suitable for the growth of fungi. The most important compounds produced by LAB and they can be used in this field are those have heat stability and they can be added to the paint or directly to the woods. Antifungal compounds synthesised by LAB can be also used to control plant disease mainly these caused by moulds. There are need to developed new fast and effective methods to identify the LAB compounds as well as the purification of these compounds to be an alternative to many of the preservation methods and chemicals.

2. CONCLUSION

Nature has developed many defence mechanisms to protect life against fungal infections. Among them are the antimicrobial compounds produced by lactic acid bacteria (LAB) such as, (lactic acid, acetic acid, peptides and phenyllactic acid) which seem to be promising in the food and medical industries. Certain strains of LAB are able to produce natural antimicrobial compounds that can inhibit the growth of spoilage and pathogen microorganisms. These compounds are varied in size and most of them are low-molecular-mass compounds, some of these compounds have been identified and many remain to be unidentified due to the lack of suitable assay and instrument. That's why; more research

should be done in antifungal area and to develop new methods to isolate even the smaller sizes of the LAB produced compounds to increase the potent to use LAB as alternative to currently used preservatives.

3. ACKNOWLEDGMENTS

This work has been supported by the Faculty of Science and Technology, University Sains Islam Malaysia.

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