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RESEARCH ARTICLE

EFFECT OF PH AND HEAT TREATMENT ON ANTIFUNGAL ACTIVITY OF *LACTOBACILLUS FERMENTUM* TE007, *LACTOBACILLUS PENTOSUS* G004 AND *PEDIOCOCCUS PENTOSACEUS* TE010

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Abstract

Lactic acid bacteria isolated from Malaysian fermented foods was evaluated for their antifungal activity. *L. fermentum* Te007 and *L. pentosus* G004 produced heat stable compounds at 90 and 121 °C while *Pediococcus pentosaceus* Te010 was heat stable at 90 °C and lost activity at 121 °C. Supernatants of *L. pentosus* G004 were active at pH between 3 and 7 while *P. pentosaceus* Te010 active at pH 5 and *L. fermentum* Te007 active at pH 3 and 7. The mixture of *L. pentosus* G004 and *L. fermentum* Te007 supernatants have high inhibition activity. Strains of lactic acid bacteria cells or their supernatants inhibited conidia germination when tested in selected foods indicating that lactic acid bacteria can be use as biopreservative.

Keywords: *Lactobacillus fermentum*; *Pediococcus pentosaceus*; *Lactobacillus pentosus; heat stability; antifungal*

Introduction

Lactic acid bacteria (LAB) produce antimicrobial compounds which can be applied as food preservatives (Stiles 1996). LABs are known to produce different antimicrobial compounds (Messens and De Vugst, 2002; Lindgren and Dobrogosz, 1990) that are inhibitory to bacteria and fungi (Hassan and Bullerman, 2008; Magnusson *et al*., 2003). *Lactobacillus pentosus* TV35b was found to have inhibitory activity against the growth of *Clostridium sporogenes*, *Cl. tyrobutyricum*, *Lactococcus. curvatus*, *Lact. fermentum*, *Lact. sake*, *Listeria innocua*, *Propionibacterium acidipropionici*, *Propioni-bacterium* sp. and *Candida albicans*. The active compound produced was a bacteriocin-like peptide (pentocin TV35b) (Okkers *et al*., 1999). 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, and inhibited the growth of *Listeria monocytogenes*.

Most of the antifungal activity studies have focused on *L. plantarum*. For example Laitila *et al*., (2002) reported that strains *L. plantarum* VTT E-78076 (E76) and VTT E-79098 (E98) inhibit the growth of *Fusarium* fungi during germination of cereals. Similarly, Magnusson *et al*., (2003) reported *L. plantarum* MILAB 14; *L. plantarum* MiLAB 393 (Ström *et al*., 2002); *L. plantarum* (Lavermicocca *et al*., 2003) produced an antifungal

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compounds that can inhibit the growth of both fungi and yeasts.

Other LAB with antifungal activity were reported, *L. coryniformis* subsp. *coryniformis* strain Si3 (Magnusson and Schnurer, 2001) and *L. paracasei* ssp. *tolerans* that completely inhibited the growth of *F. proliferatum* M 5689, M 5991 and *F. graminearum* R 4053 (Yousef and Lloyd, 2008); *L. citreum, L. rossiae* and *W. cibaria* (Francesca, *et al*., 2009). Earlier, Roy *et al*., (1996) reported *L. lactis* subsp. *lactis* CHD 28.3 inhibited the growth of *Aspergillus flavus, A. parasiticus, Fusarium* spp. Okkers *et al*., (1999) reported that *L. pentosus* inhibited the growth of *Candida albicans*.

Stiles *et al*., (1999) and Plockova *et al*., (2000, 2001) reported that *Lactobacillus* strains possess antifungal properties. *L. rhamnosus* VT1 exhibited strong antifungal properties capable of inhibiting the growth of many spoilage and toxigenic fungi including species in the genera *Aspergillus, Penicillium* and *Fusarium* (Plockova *et al*., 2001). Yousef and Lloyd (2008) also reported *L. paracasei* ssp. *tolerans* to completely inhibit the growth of *Fusarium proliferatum* M 5689, M 5991 and *F. graminearum* R 4053 compared to controls in a dual agar plate assay. Different LAB isolates (*Weissella confusa, W. cibaria, Leuconostoc citreum, L. mesenteroides, Lactococcus lactis, L. rossiae* and *L. plantarum*) identified from sourdough inhibit the growth of *Aspergillus niger, Penicillium roqueforti* and *Endomyces fibuliger* and their potential to be used as natural preservatives was suggested by Francesca *et al*., (2009).

Growth of spoilage molds such as *Aspergillus, Penicillium, Monilia, Mucor, Endomyces, Cladosporium, Fusarium,* and *Rhizopus* on food and fruits results is a major economic loss, and may be causing serious health hazard (Pitt and Hocking 1999). Many chemical preservatives that target fungi growth in food have been approved and used for many years. Recently the consumers are looking and demanding for products without chemical preservatives, and still maintain good shelf life and safe.

The antimicrobial compounds produced by LAB against bacteria and fungi are active in range of pH 3-4.5 and heat stable at 100°C (Magnusson and Schnurer, 2001, Messens, 2002; Lindgren, 1990; Lavermicocca, *et al*., 2003). Magnusson and Schnurer (2001) observed that the activity of supernatant with antifungal activity was stable during heating with maximum effect at pH 3–4.5. The LAB was identified as *Lactobacillus coryniformis* subsp. *coryniformis*, and found that this strain had strong inhibitory activity in dual agar plate assays against *A. fumigatus, A. nidulans, P. roqueforti, Mucor hiemalis, Talaromyces flavus, F. poae, F. graminearum, F. culmorum,* and *F. sporotrichoides.* The supernatant of a mixture of *Lactobacillus* spp from commercial silage inoculum was reported to reduce both mold growth and aflatoxin production by *A. flavus* (Gourama and Bullerman, 1995).

There are reports indicating that certain strains of *Aspergillus* are resistant to propionic acid (Suhr and Nielsen, 2004). *Aspergillus niger* is known to contaminate dough, processed food, canned food and bakery products. These fungi can grow even in low temperature and at pH 2.5 and it is one of the most resistant fungi (Pitt and Hocking, 1999). Recently, Kristian *et al*., (2009) reported that there are certain strains of *A. niger* able to produce mycotoxin such as ochratoxin A and fumonisin B2. *A. oryzae* has low pathogenic potential but may, like many other harmless microorganisms, grow in human tissue under exceptional circumstances. Allergic diseases primarily caused by *A. oryzae* have been reported in few cases, *A. oryzae* does not produce aflatoxins or any other cancerogenic metabolites (Peder *et al*., 1992). This fungi is not normally a spoilage agent for processed foods, however, this fungi is a common spoilage agent for fruits. Most of the antifungal capacity of LAB studied is due to the production of various compounds such as: phenyllactic acid, *L. reuteri* 1100 (Gerez *et al*., 2009), *L. plantarum* (Lavermicocca *et al*., 2000), fatty acids (Corsetti *et al*., 1998), cyclic peptides, cyclo (Phe-Pro), cyclo (Phe-OH-Pro) and reuterin (Magnusson, 2003), hydrogen peroxide and diacethyl (Messens and De Vugst, 2002).

Growth of spoilage molds such as *Aspergillus, Penicillium, Monilia, Mucor, Endomyces, Cladosporium, Fusarium,* and *Rhizopus* on food and fruits results in major economic losses, and may be causing serious health hazard (Pitt and Hocking 1999). Many chemical preservatives that target fungi growth in food have been approved and used for many years. Recently the consumers are looking and demanding for products without chemical preservatives, and still maintain good shelf life and safe.

There is a need to look for other types of LAB that can eliminate or reduce the growth of spoilage fungi in foods. The objectives of this study were to evaluate the antifungal activity of LAB isolates and to determine the effect of pH and heat treatment on the antifungal activity against *A. niger* and *A. oryzae*. Such a study simulates the heat treatment normally given to processed food.

Materials and Methods

Cultures and isolates

Three LABs studied; *L. fermentum* Te007, *P. pentosaceus* Te010, *L. pentosus* G004 were isolated from Malaysian fruits and fermented foodsThe four strains were maintained on MRS agar (Oxoid) and stored at 4 °C. The fungi *Aspergillus niger* and *Aspergillus oryzae* were obtained from the Microbiology Laboratory, Faculty of Food Science, University Putra Malaysia. The target fungi were chosen to represent potential spoilage fungi in foods especially in bakery products. Fungi were grown on Potato Dextrose agar (PDA, Oxoid) plates at 25 °C for 5 days, and stored at 4 °C. Conidia inocula were prepared by growing the fungi on PDA plates for 5 days. 20 ml of peptone water (0.1% w/v) were added and the conidia were collected after scratching the surface of the agar with sterile inoculating needle (Strom *et al.*, 2002). Conidia concentration was determined using a haemocytometer, and adjusted to $3.5x10^4$ conidia/cells per ml with sterile peptone water (0.1% w/v). The conidia suspensions were stored at 4 °C and for long term use at -20 °C with 20% glycerol.

Cell free supernatant preparation

L. fermentum Te007, *L. pentosus* G004 and *P. pentosaceus* Te010 were inoculated into MRS broth and incubated for 24 h at 30 °C (OD_{540} = 2.6). The cell free supernatant was prepared by centrifuging the broth in microcentrifuge (11500 x g for 10 min). Then the supernatant was filtrated using sterile filtered (0.45 µm-pore-size filter, Millipore).

Effect of pH treatment on LAB supernatant against fungal growth

The pH of the supernatants of the LAB isolates was adjusted to different pH values 3, 5, 6, 7 and 9 using 0.1 N HCl and/or 0.1 N NaOH. The initial pH of the isolates were 3.4, 4.6 and 4.9 for isolates (*L. pentosus* G004, *L. fermentum* Te007 and *P. pentosaceus* Te010), respectively. The adjusted pH supernatant was then tested against *A. niger* and *A. oryzae* in microtiterplate assay. 100 µl MRS broth containing 10^5 conidia/ml were placed in the 96 wells plate and 100 µl pH adjusted supernatant were added into the wells. The plates were incubated at 30 ºC for 24, 48 and 72 h. Fungal growth was measured by optical density at 560 nm (Microplate Autoreader EL 309, Biotek Instruments, Winooski, Vt.) and visually. The percentage growth of fungi was measured using the equation:

$$Percent\ growth\ of\ fungi = \frac{OD_{560nm\ after\ 48or72h} - OD_{560nm\ at\ 0h}}{OD_{560nm\ at\ 0h}} \cdot 100$$

The supernatant was heated to 90 ºC for 30 min before the experiment. All tests were done in duplicate.

Effect of heating treatment on LAB supernatant against fungal growth

The supernatants were heat treated at 90 and 121°C in the autoclave for 30 min and immediately cooled in ice water. The heat treated supernatants were then tested against *A. niger* and *A. oryzae* in microtiterplate assay. The percentage growth of

fungi was measured using same equation mentioned previously. All tests were done in duplicate.

Effect of heat treatment on antifungal activity of mixtures of supernatants

Mixtures of supernatants were tested against *A. niger* and *A. oryzae* in microtiterplate assay. The mixtures were A: *Lactobacillus fermentum* + *Lactobacillus pentosus*, B: *Lactobacillus fermentum* + *Pediococcus pentosaceus* and C. *Pediococcus pentosaceus* + *Lactobacillus pentosus*. The percentage growth of fungi was measured using same equation mentioned previously. All tests were done in duplicate.

Determination of phenyllactic acid by HPLC

The method of Valerio *et al*., (2004) was used to detect the phenyllactic acid produced by lactic acid bacteria with some modifications. Ten milliliters of cell-free supernatant was adjusted to pH 2.0 with 10 M formic acid and extracted two times with 30 ml of ethyl acetate. Anhydrous Na_2SO_4 was added to the combined organic extracts, which were then filtered through Whatman No. 1 filter paper (Whatman, Maidstone, UK) and evaporated using a vacuum rotary apparatus (Beuchi RE 111, Switzerland). The dried residue was reconstituted to the initial concentration with 10 ml of water containing 0.05 % TFA (v/v), filtered (0.22 µm, Millipore). 5 µl of the supernatant was injected into the HPLC system (Agilent 1200 series autosampler, and HP 1200 series VWD detector (210 nm). The solvent system was: solvent A (70 % acetonitrile) and solvent B (deionized water 30 %). An Isocratic HPLC separation was performed using C_{18} column (Zorbax 300SB, C18 4.6 x 250 mm 5um4.6 x 250 mm, 5 µm). The mobile phase for isocratic elution was pumped at 1 ml/min, at room temperature; detection was 210 nm. 5 µl of DL-3-phenyllactic acid was used as to develop the standard curve.

Data analysis

The survival percent of fungi measured by Optical density was used to generate the data; all tests were performed using Minitab 15 software.

Results and discussions

Effect of pH treatment on LAB supernatant against *Aspergillus niger* and *A. oryzae*

Growth of *Aspergillus niger* was observed between pH 3 to 7 but more at pH 3. The fungal inhibitory activity of *L. pentosus* G004 was observed at pH 5 and 7 but more pronounced at pH 3 (Table 1). *L. fermentum* Te007 supernatants show antifungal activity at pH 5 and 7, but *P. pentosaceus* Te010 inhibit fungal growth at pH 5. Growth of *Aspergillus oryzae* was affected by pH of growth media, little growth at pH 3 compared to 5, 6 and 7 (Table 2). All the isolates inhibit growth of the fungi at pH 7 but not pH 5 and 6. However, the supernatant of *L. pentosus* G004 and *L. fermentum* Te007 showed antifungal activity at pH 3.

Heat stability of antifungal activity of supernatant against *A. niger* and *A. oryzae*

L. pentosus G004 reduced the growth of *A. niger* about 30-fold while *L. fermentum* Te007 reduced the growth about 6-fold compared to control when the supernatant was heat treated at 90 °C for 30 min.

However, growth of fungi was observed after 72 h incubation (Figure 1and 2, Table 3 and 4). Heating the supernatant to 121 °C destroyed the antifungal activity of *P. pentosaceus* Te010.

However, the antifungal activity of the isolates *L. fermentum* Te007 and *L. pentosus* G004 increased when the supernatant were heated to 121 °C for 30 min (Figure 2 and Table 2).

Growth of *Aspergillus oryzae* was completed inhibited by the heated supernatant of *L. pentosus* G004 and *L. fermentum* Te007 at both temperatures (Figure 3, 4 Table 5 and 6). However, heating the supernatant at 121 °C destroyed the antifungal activity of *P. pentosaceus* Te010.

Table 1. *Percentage growth of Aspergillus niger in pH adjusted MRS broth containing 100 µl of the LAB supernatant*[a]

		Time (h)	
pH	**Isolate**	**48**	**72**
pH 3	*L. pentosus* G004	1.8	5.4
	L. fermentum Te007	15	49.8
	P. pentosaceus Te010	182	192
	Control	212	321.7
pH 5	*L. pentosus* G004	36.3	39.6
	L. fermentum Te007	36.5	39
	P. pentosaceus Te010	35.8	39.5
	Control	147.5	211
pH 6	*L. pentosus* G004	75.1	89
	L. fermentum Te007	156.6	231
	P. pentosaceus Te010	142.5	263
	Control	158	265.6
pH 7	*L. pentosus* G004	29.9	31.5
	L. fermentum Te007	33.5	38.6
	P. pentosaceus Te010	139.3	131.6
	Control	171.9	225.5

[a]The supernatants were adjusted to different pH using 0.1 N HCl and 1 N NaOH then heated to 90 °C for 30 min.

Table 2. *Percentage growth of Aspergillus oryzae in pH adjusted MRS broth containing 100 µl of the LAB supernatant*[ab]

		Time (h)	
pH	**Isolate**	**48**	**72**
pH 3	*L. pentosus* G004	ng	ng
	L. fermentum Te007	ng	ng
	P. pentosaceus Te010	128.8	192
	Control	88.3	147.9
pH 5	*L. pentosus* G004	44	118.6
	L. fermentum Te007	59	91.9
	P. pentosaceus Te010	4.8	185.8
	Control	105	304.6
pH 6	*L. pentosus* G004	86	105.5
	L. fermentum Te007	191	252.9
	P. pentosaceus Te010	178	296
	Control	147.8	335.6
pH 7	*L. pentosus* G004	5.5	8
	L. fermentum Te007	4.4	4.4
	P. pentosaceus Te010	0.5	2
	Control	176.5	342

[a]The supernatants were adjusted to different pH using 0.1 N HCl and 1 N NaOH then heated to 90 °C for 30 min.
[b] ng/ no growth

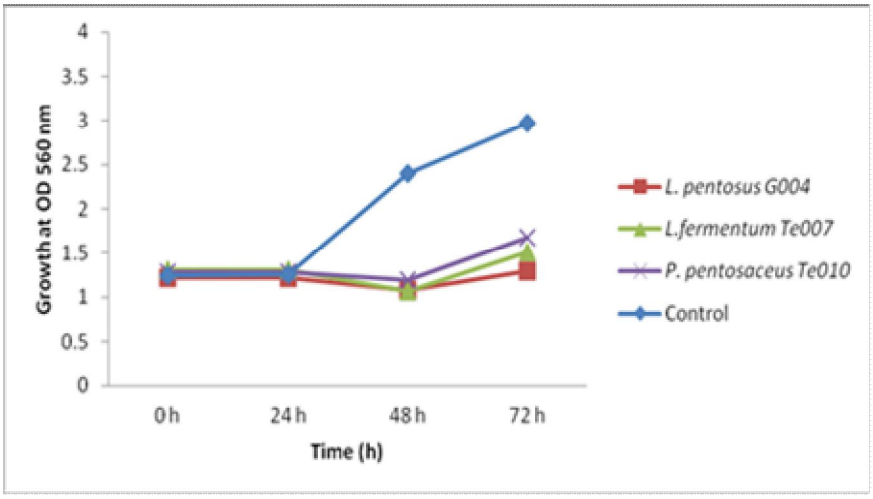

Figure 1. *Growth of Aspergillus niger in MRS broth containing 100 µl supernatant* [a]

[a] The supernatant was heated at 90 °C for 30 min and fungal growth was measured at 560 nm

Table 3. *Percentage growth of Aspergillus niger in MRS broth with different supernatants heated to 90 ºC then incubated at 30 ºC*[a]

	Time (h)	
Isolate	**48**	**72**
Control	92.6	138.7
L. pentosus G004	ng	4
L. fermentum Te007	ng	21.2
P. pentosaceus Te010	ng	35

[a] ng/ no growth

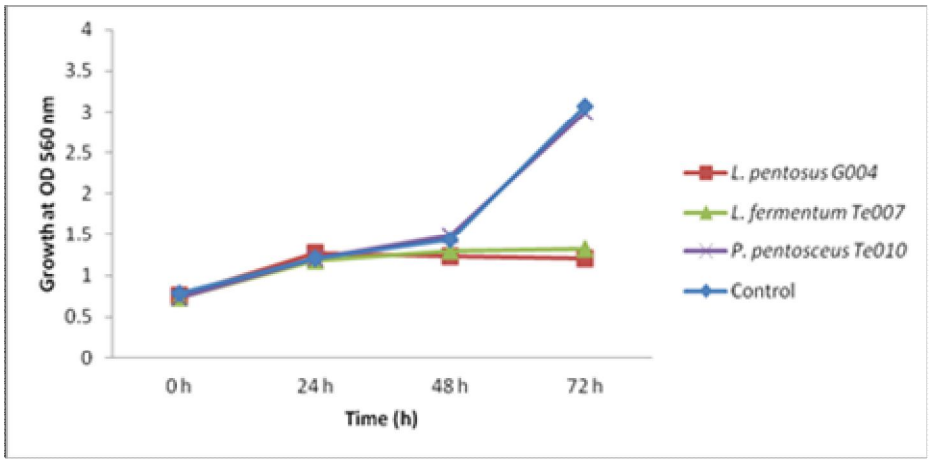

Figure 2. *Growth of Aspergillus niger in MRS broth containing 100 µl supernatant* [a]

[a] The supernatant was heated at 121 °C 30 min and fungal growth was measured at 560 nm

Table 4. *Percentage growth of Aspergillus niger in MRS broth with different supernatants heated to 121 ºC then incubated at 30 ºC*

	Time (h)	
Isolates	**48**	**72**
Control	84.6	294
L. pentosus G004	57.1	54
L. fermentum Te007	66.4	69
P. pentosaceus Te010	91.3	283

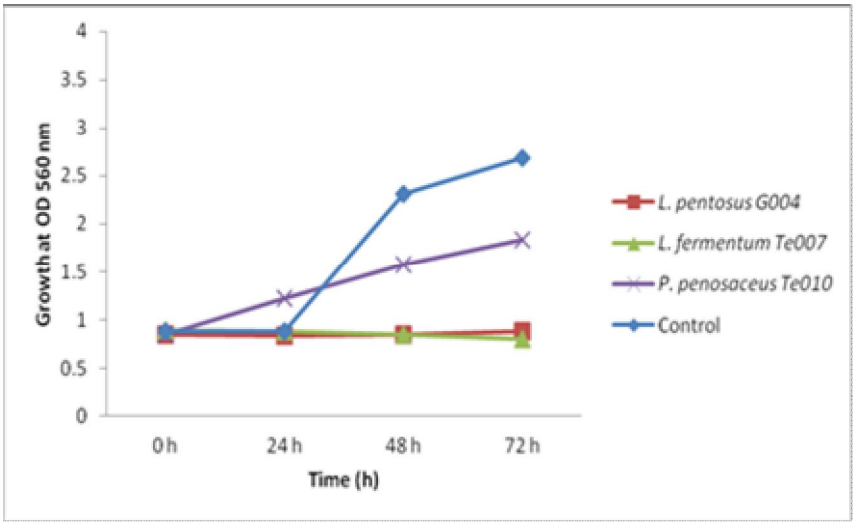

Figure 3. *Growth of Aspergillus oryzae in MRS broth containing 100 µl supernatant*[a]

[a] The supernatant was heated at 90 °C for 30 min and fungal growth was measured at 560 nm

Table 5. *Percentage growth reduction of Aspergillus oryzae in MRS broth with different supernatants heated to 90 ºC then incubated at 30 ºC*[a]

	Time (h)	
Isolates	**48**	**72**
Control	163.9	206.5
L. pentosus G004	ng	0.5
L. fermentum Te007	ng	-0.56
P. pentosaceus Te010	79.9	110

[a] ng/ no growth

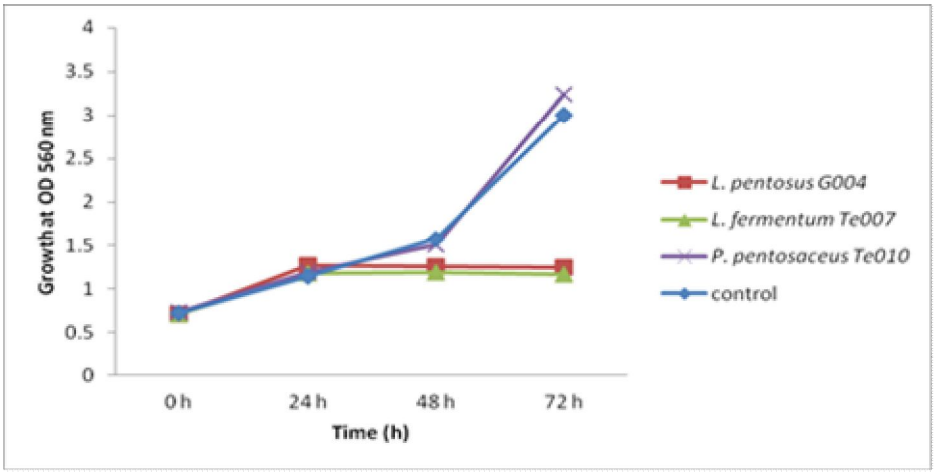

Figure 4. *Growth of Aspergillus oryzae in MRS broth containing 100 µl supernatant*[a]

[a] The supernatant was heated 121 °C for 30 min and fungal growth was measured at 560 nm

Table 6. *Percentage growth reduction of Aspergillus oryzae in MRS broth with different supernatants heated to 121 ºC then incubated at 30 ºC*

	Time (h)	
Isolates	**48**	**72**
Control	119	317.6
L. pentosus G004	73.6	72.6
L. fermentum Te007	64	62
P. pentosaceus Te010	108.3	350

Antifungal activity of combination of supernatants against *Aspergillus niger* and *Aspergillus oryzae*

The combination of mixtures of supernatant showed that mixture A (*L. pentosus* G004 + *L. fermentum* Te007) inhibited the growth of both tested fungi after 72 h at 30 °C. Mixture B (*L. fermentum* Te007 + *P.pentosaceus* Te010) inhibited the both fungi with good activity but less than mixture A.

Mixture C (*P. pentosaceus* Te010 and *L. pentosus* G004) did not inhibit the *A. niger* and *A. oryzae* as well as the other mixtures. The activity of mixture C was very weak.

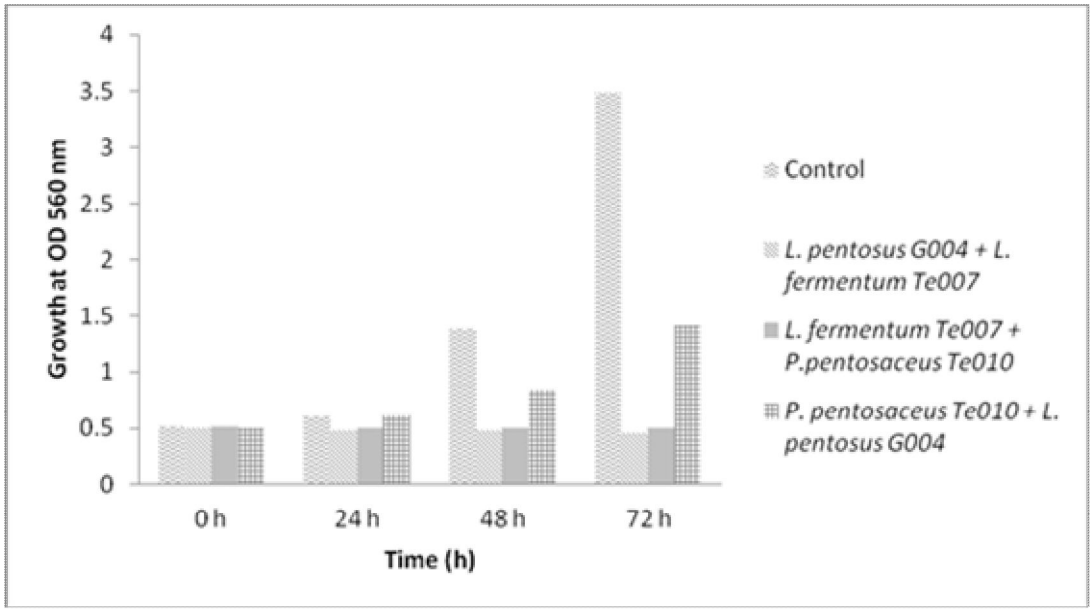

Figure 5. *Growth of Aspergillus niger in MRS broth containing 100 µl supernatant* [a]

[a] The supernatant was combined in different mixtures and heated at 121 °C for 30 min and fungal growth was measured at 560 nm

Mixing supernatants form Mixture A (*L. pentosus* G004 + *L. fermentum* Te007) completed inhibited both *A. niger* and *A. oryzae* (Figure 5 and 6). Growths of the fungi were also inhibited by mixture B (*L. fermentum* Te007 + *P. pentosaceus* Te010). Growth of the fungi was slow when mixture C (*P. pentosaceus* Te010 + *L. pentosus* G004) was used.

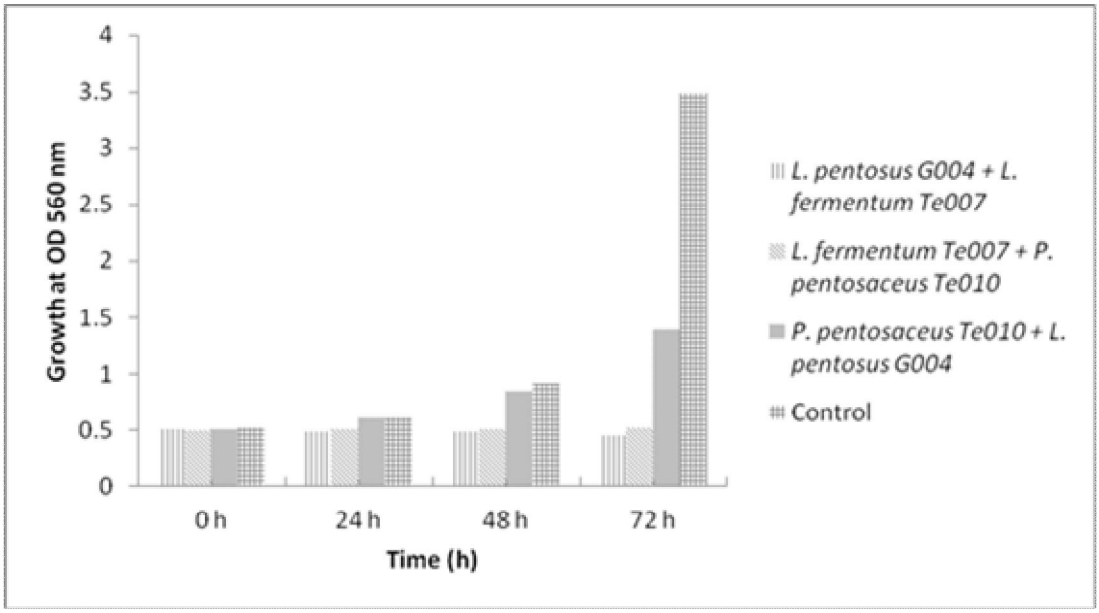

Figure 6. *Growth of Aspergillus oryzae in MRS broth containing 100 µl supernatant*[a]

[a] The supernatant was combined in different mixtures and heated at 121 °C for 30 min and fungal growth was measured at OD 560 nm

HPLC separation and evaluation of phenyllactic acid

The HPLC results showed absence of phenyllactic acid from all the isolates of LAB. The chromatogram profile showed several peaks, with variable in concentration and retention time.

In this study *L. fermentum* Te007 was observed to give good inhibitory activity against *A. niger* and *A. oryzae*, especially the latter. The heat stability study indicates the compounds are heat stable when treated at 90 and 121 °C for 30 min (Figure 1 and 2). Additionally, the antifungal activity was enhanced when the supernatant was heated to 121 °C for 30 min (Figure 4). Other workers observed that heating the supernatants of *L. coryniformis* subsp. *coryniformis* strain Si3 did not destroy the antifungal activity when treated at 100 °C for 30 min (Magnusson and Schnurer, 2001). Heating the supernatant of *L. citreum, L. mesenteroides, L. plantarum* and *L.rossiae* at 100 °C for 60 min did not destroy their antifungal activity (Francesca *et al.*, 2009). *L. pentosus* that produce pentocin TV35b, a bacteriocin-like peptide was reported to be heat stable when heated at 100 °C for 30 min but lost 50% of its activity after 15 min at 121 °C (Okkers *et al.*, 1999).

Lactic acid bacteria produce compounds that have antifungal activities and have been studied for the biopreservation of food. The antifungal activity of *L. fermentum* Te007, *P. pentosaceus* Te010 and *L. pentosus* G004 isolated from Malaysian fruits and fermented foods were affected by the heat treatment given to the supernatants and the pH of media as well as the *Aspergillus* species used. Most of the studies on antifungal activity of LAB used food spoilage fungi such as *A. fumigatus, A. nidulans, Penicillium commune, Fusarium sporotrichioides* and *Penicillium roqueforti* Magnusson *et al.*, (2003). Francesca, *et al.*, (2009) reported the antifungal activity of LAB to the fungi species *A. niger, P. roqueforti* and *Endomyces fibuliger* isolated from bakery products, while Laitila *et al.*, (2002) studied antifungal of LAB against *F. avenaceum, F. culmorum, F. graminearum and F.oxysporum* on germinated wheat.

The pH of the supernatant was adjusted to pH 3, 5, 6 and 7 before heating at 121 °C 30 min. Antifungal activity was maintained by *L. pentosus* G004 at pH 3, 5, 6 and 7; *L. fermentum* Te007 showed inhibitory activity at pH 3 and 7 while *P. pentosaceus* Te010 showed inhibitory activity at pH 5. The low pH (pH 3.7) of cultured MRS media inoculated with the bacteria further supports that organic acids are produced by this bacterium, and are responsible for the antifungal activity. Ström *et al.*, (2002) suggested that lactic acid also contribute to the inhibition of the fungi. 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.

Magnusson and Schnurer (2001) observed that *L. coryniformis* subsp. *coryniformis* had antifungal activity maximum effect at pH 3–4.5 after heat treatment at 100 °C 15 min. The antifungal activity of *L. citreum, L. mesenteroides, L. plantarum* and *L.rossiae* was gradually lost when the pH value of the supernatants was adjusted to pH values above 4. Okkers *et al.*, (1999) observed that *L. pentosus* maintain the antimicrobial activity when incubated in buffers at pH values ranging from 1 to 10. Several studies suggested that the most of the antifungal capacity of LAB studied is due to the production of organic acid (Gerez *et al.*, 2009; Corsetti *et al.*, 1998). Other researchers reported the present of phenyllactic acid together with organic acids that further enhance the antifungal activity (Lavermicocca *et al.*, 2000; and Lavermicocca *et al.*, 2003). However, phenyllactic acid was not detected from the three LAB isolates used in this study.

L. fermentum Te007 showed inhibitory activity at pH 3 and 7 while *P. pentosaceus* Te010 showed inhibitory activity at pH 5. Bacteriocins are active between pH 2 to 12 (Torodov and Dicks, 2005); Hernández *et al.*, (2005) observed *L. plantarum* TF711 showed highest antimicrobial activity at pH 1 and 9. Bacteriocins are well known to prevent the growth Gram-positive and Gram-negative bacteria but not known to inhibit fungi (Stevens *et*

al., 1991; Rodriguez, 1996; Moreno *et al*., 2000). It is possible that the supernatants contain a mixture of protein-like compounds which are not bacteriocin. The identity of the antifungal protein-like compound was not identified in this study. Different forms of cyclic peptides with antifungal activity were identified by Magnusson (2003) which were cyclo (Phe-Pro), cyclo (Phe-OH-Pro) and by Ström *et al*., (2002) which were cyclo (Phe-OH-Pro), cyclo (Phe-Pro) (Ström *et al*., 2002). The wells of micro-titter plates treated with the supernatants of *L. pentosus* G004 and *L. fermentum* Te007 and *P. pentosaceus* Te010 were clear with no visible mycelia growth. This indicates that the supernatant prevent conidia germination.

Combining *L. fermentum* Te007 and *P. pentosaceus* Te010 (Mixture B) inhibited the growth of both fungi. In contrast complete fungal growth inhibition was observed when combining (*L. pentosus* G004) and (*L. fermentum* Te007) (Mixture A). The mode of action of protein-like compounds is related to the formation holes in the membrane of the target organisms which cause leakage of cytoplasm (Todorov, 2009; Moll *et al*., 1996). The organic acids inhibitory activity against microorganism is related to the decrease of pH value. The plasma membrane of most microorganisms restricts penetration by charged molecules. However, un-dissociated molecules can easily diffuse (Stratford, 1999). It is possible that the combined effect of the protein-like compound cause pore formation of the conidia allowing the penetration of organic acid to prevent germination of conidia. Such concept is practiced in hurdle which is combination of different factors chemical and physical or combination of different compounds produced by LAB to result better biopreservation (Leisner *et al*., 1995; Helender *et al*., 1997).

Conclusions

This study shows that lactic acid bacteria isolated from Malaysian fruits and fermented foods have antifungal activity against common mould *A. niger* and *A. oryzae*. *L. fermentum* Te007 and *L. pentosus* G004 produced heat stable compounds at 90 and 121 °C. Supernatants of *L. pentosus* G004 and *L. fermentum* Te007 were active in pH between 3 and 7 while *P. pentosaceus* Te010 active at pH 5. Mixture of the isolates gave higher antifungal activity especially mixture A (*L. pentosus* G004 + *L. fermentum* Te007) which gave high inhibitory activity against the tested fungi. The supernatant of these LAB isolates has potential to be use as food bio-preservation especially in heat processed foods.

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