Research Journal of Applied Sciences 7 (9-12): 466-473, 2012
ISSN: 1815-932X

Screening of Lactic Acid Bacteria as Biocontrol Against (*Colletotrichum capsici*) on Chilli Bangi

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Abstract: Chilli (*Capsicum annuum* L.) is one of the major crops, vegetables grown in Malaysia and belongs to family Solanaceae. Pepper is suffering from many illnesses caused by fungi, bacteria and virus. The fungal disease is both seed and air borne and affect the germination of seeds and plant vital to a greater extent. Biological control by antagonistic microorganisms is widely recognized as a promising method for control plant diseases. This study reports the effectiveness of using Lactic Acid Bacteria (LAB) cultures or their supernatant as a biological control against anthracnose disease in chilli caused by fungus *Colletotrichum capsici*. From 324 lactic acid bacteria isolated from different sources, seven isolates showed good inhibition activity against *C. capsici* by Dual Overlay Method. The supernatant from LAB-C5 showed strong inhibition to fungal growth evaluated using microtitter plates. Seeds infected with *C. capsici* followed by treatment with LAB-C5 cells showed better seed germination rate than seed treated with supernatant. Fungi infected seeds fail to grow. The results indicate that LAB-C5 has potential to be used biological control against *C. capsici* to replace the use of chemical fungicide to treat chilli seeds.

Key words: Chilli anthracnose, *Colletotrichum capsici*, lactic acid bacteria, infected seeds, germination percentage

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important vegetable cash crops grown in Malaysia and belongs to family Solanaceae. Chilli suffers from many diseases caused by fungi, bacteria and virus. The disease is both seed borne and air borne and affects seed germination and vigour to a greater extent (Asalmol *et al*., 2001). Anthracnose caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has been found to be aserious disease in the chilli growing areas of Malaysia is an important disease of chilli and many kinds of plants in tropical areas. Anthracnose attacks on a wide range of plants including cereals, legumes, grasses, vegetables and fruit plants. The pathogenic fungi by *C. capsici* and *C. gloeosporioides* were reported as chilli anthracnose in Thailand (Sangchote *et al*., 1998; Sangchote, 1999). They are also the main causal in the tropical Asia (Kim *et al*., 1989). Apart from these species, *C. graminicola* and *C. atramentarium* had been reported in India (Verma, 1973) and *C. cocodes* in Florida (Roberts *et al*., 2001). *Colletotrichum* sp. is capable of causing disease on many of the chilli plant parts during any stages of growth (Kim *et al*., 1989; Sangchote *et al*., 1998).

Control of plant diseases has depended primarily upon the application of chemical fungicides despite potentially toxic effects on humans, wildlife and the environment (De Waard *et al*., 1993; Knight *et al*., 1997). The advent of new pathogen races that are resistant to fungicides has been another problem to plant pathologists and agrochemists (Delp, 1980; Staub, 1991). A microbial inoculant containing many kinds of naturally occurring beneficial microbes called effective microorganisms has been used widely in nature and organic farming (Iwaishi, 2001). Effective Microorganisms (EM) a culture of coexisting beneficial microorganism predominantly consisting of lactic acid bacteria, photosynthetic bacteria, yeast, fermenting fungi and actinomycetes are claimed to enhance microbial turnover in soil and is thus known increase soil macronutrients and

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increases plant growth and yield (Kirithiga, 2010). The application of EM has been shown to improve soil and irrigation water. It can be used in seed treatment. It can be used to make organic sprays for the enhancement of photosynthesis and control of insects, pests and diseases (Shah *et al*., 2001).

The used of chemical fungicides would caused the development of resistance by the fungal and also the risk of polluting the environments. The used of biological control agents had been suggested as an alternative way of controlling plant diseases (Compant *et al*., 2005). Selected microorganisms such as Lactic Acid Bacteria (LAB) isolated from fresh fruits and vegetables showed inhibitory activities against phytopathogenic and spoilage bacteria and fungi (Trias *et al*., 2008b) and can be used as biological control agents to protect seeds and seedlings from various pathogens (Trias *et al*., 2008b; McGee, 1996).

During the past 50 years, many studies were reported about bacterial and fungal plant diseases as well as the application of different microorganisms as biocontrol agents. However, no information is available on the interactions of LAB with phytopathogenic fungi (Compant *et al*., 2005; Heydari and Pessarakli, 2010; Hamed *et al*., 2011).

The purpose of this study was to screen LAB isolated from different sources for antifungal activity against *C. capsici* that infect chilli seeds. This study would reduce the use of chemical and use LAB as biological control to reduce plant disease and increase the growth of seeds.

MATERIALS AND METHODS

Isolation and characterization of lactic acid bacteria: Lactic acid bacteria were isolated from fresh fruits and vegetables. The vegetables (1 g) were cut into small pieces and suspended into 9 mL MRS broth (De Man *et al*., 1960) and the bags manually agitated Turbid broth was serial diluted with peptone water (0.1% w/v) from 10^{-1} to 10^{-7} on modified MRS agar with 0.8% $CaCO_3$, 0.1% (w/v) cycloheximide to inhibit possible fungal contamination. All plates were incubated under anaerobic condition in anaerobic jar at 30°C for 48 h or until the bacterial colonies are of sufficient size for colony formation. Colonies were tested for catalase activity with 4% H_2O_2. All isolates were checked for catalase negative and Gram positive reactions. Selected LAB isolates were maintain in MRS broth containing 30% glycerol and kept at -20°C. Growth conditions were determined in MRS broth at different temperatures (10 and 45°C), pH (4.4 and 9.6) and with different salt concentrations (NaCl) of 6.5 and 18% at 30°C for 48 h. Growth was determined in terms of turbidity (Sathe *et al*., 2007).

Identification of LAB by API 50 CHL kit: For species-specific identification, selected LAB strains were preliminarily identified based on the phenotypic properties such as carbon dioxide production from glucose were subjected to API 50 CHL kit assay (Bio Merieux, l'Etoile, France) following the methods described by the manufacturer (Tamminen *et al*., 2004). The inoculated strips were incubated at 30°C and then monitored for changes in the color of the medium after 1, 2 days. Change in color was represented by a positive sign (+) while a negative sign (-) represented no change. Discrimination between isolates was based on the principle of a pattern matching manual as described by the manufacturer.

Initial screening for antimicrobial activity: The inhibition activity of the isolates will be determined using the Overlay Method as described by Magnusson and Schnurer (2001). LAB will be inoculated in two 2 cm lines on MRS agar plates and grown at 30°C for 48 h in anaerobic jars. The plates will be overlaid with 10 mL of NA agar containing 10^4 resistant bacteria per mL. After 48 h of aerobic incubation at 30°C, the zone of inhibition will be measured. The scale that will be used as follows: −no visible inhibition, + no bacteria growth on 0.1-3% of plate area/bacterial streak, ++ no bacteria growth on 3-8% of plate area/ bacterial streak, +++ no bacteria growth on >8% of plate area/bacterial streak. Inhibition tests will be done in duplicated.

Screening of isolates for antifungal activity: Pure culture of *C. capcisi* was obtained from Faculty of Agriculture, University Putra Malaysia and maintained on Potato Dextrose Agar (PDA) plates and incubated at 28°C for 7 days. Selected LAB isolates were screened for antifungal activity against *C. capcisi* by overlay technique as described by Strom *et al*. (2002) on MRS agar plates. Briefly, overnight cultures of LAB were grown as two 2 cm streaks on MRS agar plate followed by incubation at 30°C for 48 h under anaerobic conditions. These plates were overlaid with semisolid malt extract agar (0.7%) seeded with 10^4 spores mL^{-1} of *C. capcisi* and incubated aerobically at 30°C for 24-72 h.

Determination of inhibitory activity on mycelia growth by well method: For preparation of culture supernatants, the randomly selected LAB isolates were grown in MRS broth at 30°C for 18 h without shaking. Cells were removed by centrifugation at 8,000 rpm for 10 min at room temperature by mini spin, eppendorf followed by filtration of the culture supernatants through a 0.45 mm pore size filter (minisart, sartorius) (Stonsaovapak *et al*., 2003) 10 mL of supernatant were placed in 50 mL flask and inoculated in triplicate with MRS broth containing 10^4 conidia/mL

of *C. capsici*. The cultures were incubated at 30°C for 24, 48 and 72 h and fungal growth was measured by OD at 560 nm (Muhialdin and Hassan, 2011).

Seeds experiment: Chilli Bangi seeds (CB) were plated in four replicates of 100 seeds and each twenty five seeds per plate were kept at 4°C before use. Firstly the seeds surfaces were disinfected with ethanol (70%) for a minute and rinsed with distilled water four times to minimize microorganism development at the early stages of germination (Kurtar, 2010). The treatments included immersion of seeds for 10 min in LAB-C5 and LAB-C5S prepared on MRS Broth (10^8 cfu mL^{-1}) with 4 replicates of 25 seeds each. After immersion, The seeds were dried under laminar flow and then put them in pitri dish and some of seeds were follow by used drop of spores of *C. capsici* (10^4 spores mL^{-1}) prepared by growing the fungus on PDA for 7 days at room temperature on the seeds treated with LAB strain and let them for 7 days to observed the germination percentage and 11 days for observe the radical and plumule length For seeds as control treatment by immersion on distilled water in the same time.

Statistical analyses: All data were analysed by one-way Analysis of Variance (ANOVA) and by used Tukey test, the statistical significance ($p \leq 0.05$) program from Minitab 16 Software.

RESULTS AND DISCUSSION

Isolation and characterization of lactic acid bacteria: Seven from 150 samples of LABs isolated from fresh fruits and vegetables. All the isolates were catalase negative and Gram-positive bacteria. MRS agar with 0.8% $CaCO_3$ showed the highest number of isolates from all samples showed strong inhibitory activity against *C. capsici* (Table 1). Six LAB isolates could grow at 10 and 45°C, pH 4.4 and 9.6 and at 6.5 and 18% NaCl except isolate G1 strain which failed to grow at pH 4.4 and 9.6.

Identification of LAB by API 50 CHL assay: Phenotyping of lactic acid bacteria using biochemical (API 50CHL) in (Table 2), showed that six isolates as *Lactobacillus plantarum* and one isolate as *Lactobacillus parasasei* (Table 3).

Initial screening for antimicrobial activity: Total 7 isolates of LABs evaluated screening for antimicrobial activity as (D1, C5, G7, D10, D11,G1 and B3) showed high activity (15-23 mm) against all pathogens bacteria except D11 showed low activity (9 mm) against (Klebsilla, *E. coli*, *S. aureous*, *Streptococcus*). No bacteria growth on 3-8% of plate area/bacterial streak (Table 4 and Fig. 1).

Screening for antifungal activity against *Colletotrichum capsici* by Overlay Method: Seven of LAB isolates (D1, C5, G7, D10, D11, G1 and B3) showed good inhibitory activity against conidia germination of the fungi *C. capsici* in the Overlay Method (Table 5 and Fig. 2). Out of 5 isolates (D1, C5, G7, D10 and D11), (identified as *Lactobacillus plantarum*) had strong activity (+++ and ++++) against spoilage fungi *C. capsici* were inhibition area per bacterial streak was >10-18 mm or >20 mm of the Petri dish and then were 4 of isolates (Te, G1, D3 and B3) had moderate activity (inhibition area per bacterial streak was 6-10 mm of Petri dish) against *C. capsici*.

Fig. 1: LAB-C5 strain showing inhibitory potential against Klebsilla and *E. coli*

Table 1: Isolation and characterization of the 7 isolates of lactic acid bacteria having high inhibitory ability[a]

						Temperature (°C)		pH		NaCl (%)	
Strains	Source	Shape	Gram reaction	Catalase test	Gas production	10	45	4.4	9.6	6.5	18
B3	Dragon	Cocci	+	-	-	+	+	+	+	+	+
D1	Dragon	Rod	+	-	-	+	+	+	+	+	+
D10	Starfruit	Rod	+	-	-	+	+	+	+	+	+
D11	Melon	Rod	+	-	-	+	+	+	+	+	+
G1	Guava	Rod	+	-	-	+	+	--	+	+	+
C5	Durian	Rod	+	-	-	+	+	+	+	+	+
G7	Ginger	Rod	+	-	-	+	+	+	+	+	+

[a]Growth (+) and no growth (-)

Determination of inhibitory activity on mycelia growth by well method: The growth of the mycelia and the conidia were inhibited by the supernatant of the LAB isolates by the well method, the lowest line is the distribution column

Table 2: Identification of LAB by API 50 CHL assay

LAB isolates	Percentage	Identification
D1	99.2	*Lactobacillus parasasei*
D10	99.9	*Lactobacillus plantarum*
D11	99.9	*Lactobacillus plantarum*
G1	99.9	*Lactobacillus plantarum*
B3	95.9	*Lactobacillus plantarum*
C5	95.9	*Lactobacillus plantarum*
G7	95.9	*Lactobacillus plantarum*

Table 3: Carbohydrate fermentation profile of lactic acid bacteria isolate

Characteristic	D1	D10	D11	G1	B3	C5	G7
Glycerol	-	-	-	-	-	-	-
Erytritol	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-
L-arabinose	-	+	+	+	+	+	+
Ribose	+	+	+	+	+	+	+
D-xylose	+	-	-	-	+	+	+
L-xylose	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-
β metyl xyloside	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+
D glucose	+	+	+	+	+	+	+
D fructose	+	+	+	+	+	+	+
D mannose	+	+	+	+	+	+	+
L-sorbose	-	-	-	-	_	-	-
Rhamnose	-	?	?	-	+	+	+
Dulcitol	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-
Manitol	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+
α metyl-D-manoside	-	+	+	+	+	+	+
α metyl-D-glucoside	-	-	-	-	+	+	?
Nacetylglucosamine	+	+	+	+	+	+	+
Amygdaline	-	+	+	+	+	+	+
Arbutine	-	+	+	+	+	+	+
Csculine	+	+	+	+	+	+	+
Salicine	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Melibiose	-	+	+	+	+	+	+
Saccharose	?	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+
Inuline	-	-	-	-	-	-	-
Melezitose	+	+	+	+	+	+	+
D- raffinose	-	+	+	+	+	+	+
Starch	-	-	-	-	?	-	-
Glycogene	-	-	?	?	-	-	-
Xylitol	-	-	-	-	-	-	-
β gentibiose	-	+	+	+	+	+	+
D-turanose	+	-	-	+	+	+	+
D-lyxose	-	-	-	-	-	-	-
D-tagatose	+	-	-	-	+	+	+
D-fucose	-	-	-	-	-	-	-
L-fucose	-	-	-	-	-	-	-
D-arabitd	-	-	-	-	-	-	-
L-arabitd	-	-	-	-	-	-	-
Gluconate	?	+	+	+	?	+	+
2 ceto-gluconate	-	-	-	-	-	-	-
5 ceto-gluconate	-	-	-	-	-	-	-

+ = Positive, - = Negative, ? = Doubtful

for the supernatant of LAB-C5 (*Lactobacillus plantarum*) (Fig. 3) the isolates showed good inhibition to fungal

Table 4: LAB isolates showing antimicrobial activity against pathogens bacteria

	Pathogens of bacteria					
Strains	*S. aureus*	*E. coli*	Klebsilla	Streptococcus	Protous	*B. subtillus*
D1	+++	+++	+++	+++	+++	+++
C5	+++	+++	+++	+++	+++	+++
G7	+++	+++	+++	+++	+++	+++
B3	+++	+++	+++	+++	+++	+++
D10	+++	+++	+++	+++	+++	+++
D11	++	++	++	++	+++	+++
G1	+++	+++	+++	+++	+++	+++

Table 5: Selected lactic acid bacteria isolates inhibitory activity on *Colletotrichum capsici*, conidia germination after 48 h incubation at 30°C by Dual Agar Overlay Method

LAB isolates	*C. capsici*[a]	Sources
D1	+++	Dragon
C5	++++	Durian
G7	++++	Ginger
D10	+++	Papaya
D11	+++	Star fruit
G1	+	Guava
B3	++	Dragon

[a]antifungal activity: + = inhibition zone of>6 mm, ++ = inhibition zone of 6-10 mm, +++ = inhibition zone of 10-18 mm, ++++ = inhibition zone of >20 mm

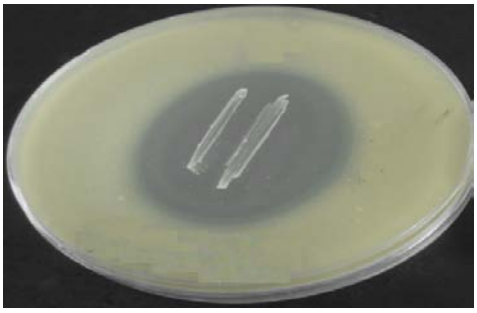

Fig. 2: Clear zone indicates growth inhibition of *Colletotrichum capsici* by lactic acid bacteria (C5) incubated at 30°C for 72 h by Dual Agar Overlay Method

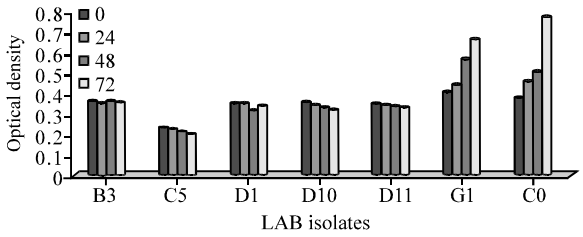

Fig. 3: Inhibition of *Colletotrichum capsici* conidia germination by LAB supernatant using agar well-diffusion assay incubated at 30°C for 72 h

growth against *C. capsici* on microttiter plates. The LAB isolates with *C. capsici* at the time (48 and 72 h) were significant (<0.005) (Table 6).

Seeds germination: There were significant differences among treatments in the germination percentage of chilli seeds (Table 7). Both the cells and supernatant LAB-C5 and LAB-C5S can be used together to inhibit the growth of *C. capsici* also the control treatment showed the greatest number of germinated seeds. Treating chilli seeds with LAB-C5 cell gave high germination percentage of CB chilli plant which was 96%, compared with LAB-C5S supernatant which gave 84.75%. Slight reduction in percent germination was observed when seeds were treated with the supernatants.

Seeds infected with the fungus *C. capsici* resulted in low percent germination at 25.25% on CB chilli seeds. The study indicates if the seeds were treated with LAB, they can resist the fungal infection as indicated by the percent germination which was similar to the LAB treated seeds. Also LAB-C5 was gave high growth for rdical and plumule length on last day (2.9 and 2.84 cm), respectively compared with control which was (2.34 and 2.28 cm). (Table 8, 9 and Fig. 4).

Table 6: Antifungal activity (%) with LAB isolates at different times

	Different times			
LAB isolates	0	24	48	72
B3	0.365±0.09	0.354±0.18	0.363±0.09	0.359±0.23
C5	0.233±0.09	0.225±0.19	0.217±0.10	0.204±0.09
D1	0.353±0.10	0.358±0.02	0.316±0.06	0.339±0.09
D10	0.361±0.01	0.343±0.05	0.331±0.10	0.318±0.01
D11	0.354±0.09	0.347±0.09	0.34±0.080	0.334±0.01
G1	0.405±0.17	0.444±0.25	0.574±0.27	0.665±0.35
Control	0.372±0.10	0.459±0.35	0.512±0.18	0.775±0.19

LAB = Lactic Acid Bacteria, at p≤0.005. The results are mean values of triplicate determinations±SD

Table 7: Percent germination of infected chilli seeds with *C. capsici* treated with LAB

Treatments	Germination percentage on Chilli Bangi (CB)
LAB-C5	96±0.7300
LAB-C5S	84.75±1.13
LAB-C5+C.C	90.75±0.86
LAB-C5S+C.C	87.25±0.86
C.C	25.25±9.49
Control	98.5±1.160

CB = Chilli Bangi, LAB-C5 = Lactic Acid Bacteria Cell, LAB-C5S = Lactic Acid Bacteria Supernatant, C.c = *Colletotrichum capsici.* results are mean values of four replicates determinations±SD

Table 8: Effect of seeds treatment with *Colletotrichum capsici* and LAB on radicle length of Chilli Bangi

	Radicle length on CB				
Treatments	7	8	9	10	11
LAB-C5	1.64±0.15	1.94±0.08	2.18±0.13	2.52±0.14	2.9±0.200
Control	1.32±0.26	1.56±0.23	1.8±0.220	2.02±0.21	2.34±0.20
LAB-C5+C.c	0.6±0.150	0.86±0.11	1.2±0.180	1.44±0.13	1.74±0.19
C.c	0.14±0.05	0.24±0.05	0.48±0.08	0.58±0.08	0.64±0.08

Table 9: Effect of seeds treatment with *Colletotrichum capsici* and LAB on Plumule length of Chilli Bangi

	Plumule length on CB				
Treatments	7	8	9	10	11
LAB-C5	1.6±0.150	1.88±0.13	2.18±0.13	2.5±0.070	2.84±0.05
Control	1.24±0.43	1.48±0.47	1.72±0.39	1.98±0.37	2.28±0.37
LAB-C5+C.c	0.7±0.150	1.02±0.13	1.36±0.08	1.58±0.08	1.92±0.19
C.c	0.14±0.05	0.26±0.08	0.42±0.10	0.48±0.08	0.54±0.05

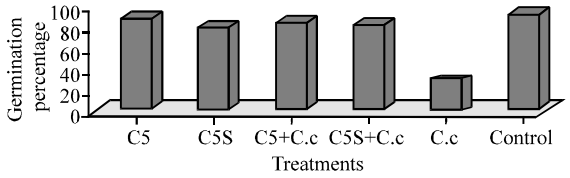

Fig. 4: Effect of seed treatment with *Colletotrichum capsici*, LAB cell and supernatant on seeds germination

Lactic acid bacteria isolated from vegetables and fruits which had good activity against *C. capsici* were tested for different temperatures, pH and NaCl which were out of 7 isolates showed strong activity against *C. capsici* (Table 1) could grow at 10 and 45°C, pH 4.4 and 9.6 and at 6.5 and 18% NaCl except G1 strain which couldn't grow at pH 4.4 and 9.6. In this study agreement with (Sathe *et al*., 2007), reported that observed their growth at extreme pH (4.4 and 9.6) and temperature (10 and 45°C), these are exciting properties with an application point of view. Also, described *L. coryniformis* subsp. *coryniformis* strain Si3 with a broad inhibitory spectrum against moulds and yeasts (Magnusson and Schnurer, 2001) also researchers found out that six isolates as *Lactobacillus plantarum* 95.9-99.9% (Table 2) and one isolate as *Lactobacillus parasasei* 99.2%.

In vitro antagonistic assays revealed that 7 of LABs strains (D1, C5, G7, D10, D11, G1 and B3) had good antagonistic activity against all phytopathogens bacteria as *Streptococcus*, *Bacillus subtillus*, *Protous*, *Klebsilla*, *E. coli* and *S. aureous* (Table 4). A earlier research using the same isolates showed that most of the selected strains had good antagonistic activity against the pathogens including *Listeria monocytogenes*, *Salmonella thyphimurium* and *Escherichia coli* (Trias *et al*., 2008a) and some of them have low active (7-9 mm). These LAB are facultative anaerobic Gram-positive bacteria, vary in their processing of fermentation, hydrogen peroxide and bacteriocin production. They also can be colonized easily *in vivo* (Alander *et al*., 1999), the combination of different organic acids such as lactic and propionic has been reported to have a synergistic fungistatic effect (Adams and Hall, 1988).

Five isolates (D1, C5, G7, D10 and D11), showed good inhibition against *C. capsici* (Table 5, Fig. 2). The results similar to study by Sathe *et al.* (2007) found suspension of *Lact. plantarum* was indicated delay in growth for *Aspergillus flavus*, *F. graminearum*, *R. stolonifer* and *B. cinerea* on cucumber. This study evaluated the efficacy of LAB isolated from fresh fruits and vegetables as biocontrol agents against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris*, *Erwinia carotovora*, *Monilinia laxa* and *Botrytis cinerea* on apple fruits (Trias *et al*., 2008b).

Very few *in vitro* studies have been reported about the efficacy of LAB against phytopathogenic fungi (Zulpa *et al*., 2003; Trias *et al*., 2008b; Wang *et al*., 2011). On the other hand the supernatant from LAB-C5 showed strong inhibition to fungal growth on microtiter plates on *C. capsici* (Table 6) which LABs isolates were significant (<0.005) at 48 and 72 h. Laitila *et al*. (2002) and Lavermicocca *et al*. (2003) suggested that the antifungal activity of *L. plantarum* could be the results of many organic acids such as lactic acetic and phenyllactic acids.

The results similar to study by Sathe *et al.* (2007) found suspension of *Lact. plantarum* was indicated delay in growth for *Aspergillus flavus*, *F. graminearum*, *R. stolonifer* and *B. cinerea* on cucumber. The conidia germination is the growth stage that is most sensitive to inhibition. In fact, the precise mechanism of antimicrobials can often not be defined because of a complex interaction between the different compounds produced during cell growth and the frequently synergistic effects among them (Legan, 1993). Another study that found suggesting that some soluble compounds in culture supernatant may be responsible for the inhibition (Tang *et al*., 2010).

LAB with antifungal and antibacterial activity are well documented in food, meat and milk products as biopreservatives (Schnurer and Magnusson, 2005) while less attention has been paid to exploit the antifungal activity of LAB for biocontrol of phytopathogenic fungi. When this effect was reported, under *in vitro* assays, it was attributed to the production of indol acetic acids and phenolic substances (Zulpa *et al*., 2003), organic acids (Trias *et al*., 2008b) or proteinaceous compounds (Wang *et al*., 2011).

LAB-C5 gave seeds resistant to fungus *C. capsici*, LAB-C5cell and LAB-C5S supernatant promote seed germination (Table 7). In this results were the treatment of LAB gave different results for germination percentage of Chilli Bangi (CB). Both the cells and supernatant of LAB can be used together to inhibit the growth of *C. capsici* also, the control treatment showed the greatest number of germinated seeds and the seeds infected with the fungus *C. capsici* resulted in low percent germination and the seeds surface were infected by growth the spores of *C. capsici* from these studies that *C. capsici* was borne intraembryonal in chilli seeds. Disruption of seed tissues could be due to the activity of cellulolytic and pectionlytic enzymes produced by *C. capsici* (Sariah, 1980). Formation of the acervulus was initiated below the seed coat and also in the endosperm and emerged to the surface after disrupting the seed coat. Parenchymatous tissues were also distorted. The pathogen finally grows on the seed surface (Sariah and Nik, 1988). Inhibitory activity of *Lact. plantarum* against fungal species are in agreement with earlier studies (Lavermicocca *et al*., 2000; Magnusson *et al*., 2003). Also, Mehrotra and Aggarwal (2003) reported that pathogenic seed-borne fungi could seriously retard seed germination through softening and necrosis of tissues. Rose *et al.* (2003) reported that two applications of the biocontrol agents *Trichoderma harzianum*, *Pseudomonas chlororaphis* and *Streptomyces griseoviridis* were needed every 10 days for effective controlling against *F. oxysporum*.

This study evaluated the efficacy of LAB isolated from fresh fruits and vegetables as biocontrol agents against the phytopathogenic and spoilage bacteria and fungi, *Xanthomonas campestris*, *Erwinia carotovora*, *Monilinia laxa* and *Botrytis cinerea* on apple fruits. (Trias *et al*., 2008b). Indicate that the induction of resistance may be another mechanism by which the yeast antagonist suppresses *C. capsici* on chilli fruits (Nantawanit *et al*., 2010). Also found that tomato seeds treated by lactic acid bacteria has been reduced the growth of *Fusarium oxysporium* and improved the growth of roots (Hamed *et al*., 2011).

CONCLUSION

Selected strains of local LAB isolates could inhibit the growth of spoilage fungi *colletotrichum capsici*. Strain of LAB cell or the supernatants could be used to inhibit spore germination, mycelia growth, germinates of seeds and spore formation of spoilage fungi.

ACKNOWLEDGEMENTS

The researchers would like to thank the Dean of Faculty of Science and Technology, Universiti Sains Islam Malaysia for making this research possible. Also, researchers thank Mansor Salem from UKM for the statistical analyses of data and Mohd Nazri and Asmalina from University Putra Malaysia for their helpful suggestions.

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