CHAPTER III

ISOLATION, IDENTIFICATION OF LACTIC ACID BACTERIA AND ITS INHIBITORY ACTIVITY AGAINST *Fusarium* SPECIES

3.1 INTRODUCTION

Lactic acid bacteria are easily isolated from fermented foods, but novel sources of LAB strains isolated from the soil can be used to control soil borne phytopathogenic fungi (Kohl et al., 2011; Matthias et al., 2012). The LAB produces a variety of antimicrobial compounds such as lactic acid, acetic acid, propionic acids, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide (Ouwehand, 1998). The use of LAB is not limited to the production of fermented foods but can be used as biocontrol agent in plants. *In vitro* studies indicated that LAB has a potential application as biocontrol against pytopathogenic fungi (Stephane et al., 2005; Rosalia et al., 2008; Ashgar & Mohammad, 2010; Wang et al., 2011; El-Mabrouk et al., 2012). Plockova et al. (2000; 2001) and Stiles et al. (1999) reported that *Lactobacillus* strains possess inhibitory properties against several fungi species. For example, *Lb. rhamnosus* VT1 showed strong antifungal properties to inhibit the growth of many spoilage and toxigenic fungi include species of genera *Fusarium, Aspergillus* and *Penicillium* (Plockova et al., 2001; Yousef & Lloyd, 2008). Growth of spoilage molds such as *Aspergillus, Penicillium, Monilia, Mucor,*

Endomyces, Cladosporium, Fusarium species, and *Rhizopus* species cause major economic loss in food and fruits, and may be causing serious health hazard (Pitt & Hocking, 1999). The economic losses and the health hazards of the mycotoxins produced by spoilage pathogens fungi are the main alarms in food industry (Gray & Bemiller, 2003). *Aspergillus* and *Penicillium* species are the most widespread spoilage fungi for many foods and feeds while *Fusarium* species are reported to invade the cereal grains in the field (Samson et al., 2004). *Fusarium* species could be identified based on growth characteristics on PDA, the fungus formed hyaline, branching mycelium that was white, gray to light pink in color. All phytopathogenic *Fusarium* isolates rendered colonies with conidia and mycelia with morphological characteristics typical for *Fusarium* species (Burgess et al., 1994). The aerial mycelium appears white and may subsequently change in color ranging from gray to violet and dark purple depending on the strain (or special form) of *Fusarium* species (Smith et al., 1988).

Fusarium species are ubiquitous in soils and are considered as field fungi invading more than 50% of maize grains before harvest and these species cause root rot. This pathogen also attack beans at all growth stages and cause damping-off at the seedling stage, yellowing of the leaves, stunted growth, and death when the population of pathogens is high in soil (Robledo-Robledo, 1991). The causative agent of *Fusarium* root rot diseases is *F. solani* (Abu-Taleb et al., 2011) infected many vegetable crops worldwide and decrease the quantity and quality of major world crops and others economical plants (Parveen et al., 1993; Ghaffar, 1995), because of *Fusarium* species are main cause of plant diseases that reported in many countries namely, in Italy (Polizzi et

3.2 MATERIALS AND METHODS

3.2.1 Isolation and characterization of lactic acid bacteria

Lactic acid bacteria were isolated from Malaysian soil, rhizosphere soil and fermented chilli fruits of different plant species according to the method of Matthias et al. (2012). Approximately, more than 300 soil samples were collected from different parts in Malaysia such as Nilai land scape garden, Local market in Nilai Seramban; Subang Jaya nursery and Puchong, Selangor. One gram of soil samples were transferred to a 50 mL Falcon-tube containing 30 mL of De Man Rogosa and Sharpe Broth (MRSB Oxoid, CM0359) and incubated in an incubator shaker (SASTEC) for 2 d at 37°C. Appropriate dilutions were prepared using sterile 0.1% (w/v) peptone water (Oxoid, CM0009), and 100 µL of dilution were spread on modified De Man, Rogosa and Sharpe agar (MRSA Oxoid, CM0361) supplemented with 0.7% $CaCO_3$ and incubated under anaerobic conditions at 37°C for 24 h (De Man et al., 1960; Wanchai et al., 2007). Colonies that showed clear zones were selected and streaked on MRS agar plates to obtain pure colonies. These colonies were tested for catalase activity with 4% H_2O_2. All isolates were checked for catalase negative and Gram positive reactions. The 24 h pure LAB isolates in MRS broth were stored at 4°C for working culture or -20°C with 20% glycerol for long term storage.

3.2.2 Determination of lactic acid bacteria growth in MRSB

Overnight culture of selected LAB (100 µL) was inoculated in 20 mL MRSB and the growth was determined by measuring the optical density (OD) at 630 nm using Nanophotomerter-P330 (IMPLEN) and Micro plate Auto reader EL 309 (Biotek Instruments, Winooski, Vt.). Based on highest growth optical density (OD) at 630 nm LAB isolates were selected for futher study namely, LAB-MSS1, LAB-SS5 isolated from soil samples, LAB-FF11 from fermented chilli fruits and IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, from ATCC culture collected from laboratory, Faculty of Science and Technology, Universiti Sains Islam Malaysia Nilai, Malayia.

3.3 Isolation phytopathogenic fungi culture

Four phytopathogenic fungi were isolated from infected plant samples including chilli leaves (CL), chilli seeds (CS), chilli fruits (FC), rose leaves (LR) collected from Nilai local areas, Seramban in Malaysia. Isolation of fungi from chilli samples was done according the method of Jha (1995). Chilli samples were washed with distilled water and patted dry with tissue paper, followed by surface-sterilize in 1% sodium hypochlorite for 2 min, before placing aseptically placed on potato dextrose agar (PDA Oxoid, CM0139) using sterilized forceps. The plates were incubated at room temperature 28°C for 5 to 7 d. Then, the colonies of different shape and colors were sub-cultured on PDA incubated at room temperature 28°C for 5 to 7 d, and pure culture of each colony was maintained on PDA and stored at 4°C. The fungi culture was also maintained as conidial spore

suspension in malt extract broth (MEB, Oxoid) with 25% glycerol and stored at -80°C as described by Judith et al. (2006).

3.3.1 Preparation of spore suspensions of phytopathogenic fungi

Phytopathogenic fungi were grown on PDA incubated at room temperature 28°C for 7 d. Sterilized distilled water (10 to 20 mL) was poured onto the plates, the fungal surface was gently scraped to loosen the spores and the spore suspensions was collected according to the method of El-Mabrouket (2014) with modification using serial dilution to obtained spore suspension at concentration of 1×10^5/mL was prepared before each experiment.

3.3.2 Measurment of growth of phytopathogenic fungi in MEB and MRSB medium

Optical densities of phytopathogenic fungi were observed in 20 mL MEB and MRSB broth inculated with 100 µL of five d old phytopathogens. Growth of phytopathogenic fungi was observed after 5 d incubation at 30°C using Nanophotomerter-P330 (IMPLEN) at OD630 nm and micro plate auto reader EL 309 (Biotek Instruments, Winooski, Vt.). Optical density was observed after 24, 48, and 72h at 30°C incubation.

3.3.3 Microscopic observation of spores and mycelia

One drop of cotton blue in lacto-phenol stain was placed on clean slide with the aid of a mounting needle a small portion of the mycelium from the fungal cultures was removed

and placed in a drop of lacto-phenol stain. The mycelium was spread well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles (James & Natalie, 2001). The morphology of the fungi was observed using light microscope (Nikon).

3.4 Preparation of cells free supernatant from lactic acid bacteria

The LAB isolates were inoculated into MRS broth and incubated for 24 h at 37°C. The LAB-CFS was prepared by centrifuging the broth at 11500 × rpm for 10 min at 4°C (Centrifuge Combi-514R, Korea). The supernatant of each LAB isolates was filtrated using sterile filtered 0.45 µm pore size Millipore filter (Schleicher and Schuell, Dass el, Germany). Then, LAB-CFS were evaluted for antifungal activity against *Fusarium* species where isolated from different plant part using the qualitative and quantitative methods.

3.5 Qualitative evaluation of antifungal activity of lactic acid bacteria against phytopathogenic fungi

Three different methods were used for screening antifungal activity by LAB cell or LAB-CFS against targated phytopathogenic fungi *Fusarium* species which were dual overlay, well diffusion, and on potato dextrose agar (PDA).

3.5.1 Dual overlay method

Inhibition activity of the LAB isolates against phytopathogenic fungi was determined using the overlay method as described by Magnusson and Schnurer (2001). Initially, LABs were inoculated in 2 cm lines on MRS agar plates and was aerobically incubated at 37°C for 24 h. The plates were then overlaid with 10 mLof malt extract semi solid agar (0.07%) (Oxoid) containing 1 mL spores (10^7/mL) suspension of targeted pathogenic fungi. After 48 h of aerobic incubation at 37°C, the zone of inhibition was measured using the general scale and inhibition tests were done in duplicate.

3.5.2 Well diffusion method

The LAB isolates which showed antifungal activity was further tested using the well method as described by Magnusson and Schnurer (2001). Seven days old fungi grown in MEB were used and 1 mL spores suspensions (10^5/mL) were mixed in 9 ml sterile distilled water. Next, 50 µL of spores suspensions were spread on MEA agar and allowed to dry for a few minutes in laminar flow cabinet. Then, wells of size 7 mm were made using sterilized cork borer and 1 to 2 drops MEA agar was pipetted to cover the base of the well to avoid leaking of the supernatants. Filtered of sterilized CFS (200 µL) were added to each well and the plates were incubated at 30°C for 24 h. Diameter of mycelia growth inhibition was measured in millimeter (mm) and were done in triplicate.

3.5.3 Inhibition on potato dextrose agar

Selected LAB isolates was tested against fungi using the PDA method was done according to the method of (Nene & Thapliyal, 1973) with modifications. The CFS (1 mL) was mixed with 100 mL PDA, and distributed to five petri dishes. After the agar has solidified it was inoculated with mycelia plugs with the aid of tungsten wire loop and the plates were incubated at room temperature 28°C for 7 d. Diameter of fungal growth was recorded and the percentage of inhibition of mycelia growth was calculated by using the formula as described (Vincent, 1947) and were done in duplicate.

$$[GI\%] = [TC-TT/TC] \times 100$$

Where,

GI% = growth inhibitions,

TC = fungal mycelium on PDA (as control),

TT = fungal mycelium on PDA treated LAB-CFS (as treatment samples)

3.6 Quantitative evaluation of lactic acid bacteria antifungal activity against phytopathogenic fungi by different methods

Two different methods were used for evaluating antifungal activity of LAB cells or supernatants against phytopathogenic fungi which were dry method by LAB cells in MRSB, dry method by LAB-CFS in MEB as below using the formula described by Hamed et al. (2011).

3.6.1 Dry method by LAB cells in MRSB

Spore suspension (100 µL) of seven days old fungi was obtained with serial dilution method (1×10^5 spores/mL) were inoculated to 20 ml MRSB and added with 24 h LAB cells (100 µL). The bottles were incubated in orbital shaker (Protech Orbital Shaker) at 150 rpm at room temperature 28°C for 7 d. After that fungal mass were filtered, washed several times with distilled water, and dried at 55°C in drying oven (BINDER) until a constant weight. The quantitative antifungal activity by cells of LAB was determined following the method of Hamed et al. (2011) with modifications. Percentage inhibition of mycelia growth was calculated using the formula given below.

$$[GI\ (\%)] = [TC\text{-}TT/TC] \times 100$$

Where,

GI (%) = growth inhibition

TC = total biomass in control

TT = total biomass in treatment

3.6.2 Dry method by LAB supernatants in MEB

In vitro assays for quantitative antifungal activity by LAB were determined following the method of Hamed et al. (2011) with modifications using conical flasks (250-mL) containing 40 mL MEB as the growth medium for all the tests fungi. LAB-CFS (5 mL) were inoculated with the test fungi (1×10^5 /mL) and incubated in orbital shaker (Protech

Orbital Shaker) 150 rpm at room temperature for 7 d. After incubation for fungal growth were filtered, washed several times with distilled water, and dried at 55°C to a constant weight. Percentage growth inhibition (GI%) was calculated using the formula as described in section 3.6.1.

3.7 Identification of LAB Isolates

The LAB isolates were identified by phenotypically using API CH 50 kit and genotypically using polymerase chain reaction.

3.7.1 Phenotypic identification of LAB isolates using API CH 50 kit

The LAB isolates that showed the strongest antifungal activity were identified using API 50 CH kit (API system, Bio Merieux, France) following the methods described by the manufacturer. Strips were incubated at 37°C as recommended by the manufacturer. Changes in colors either to yellow or blue were recorded after 24 and 48 h and the results were analyzed using API WEB (Bio Merieux).

3.7.2 Genotypic identification of LAB isolates using polymerase chain reaction

The isolates of LAB were that showed strong antifungal activity was selected for genomic identification based on method described by Jarvis and Hoffman (2004).

3.7.3 DNA extraction of LAB isolates

An overnight culture cells were used for total genomic DNA extraction in 20 ml MRS broth at 37°C using Master Pure™ Gram positive DNA purification Kit (USA). One ml of overnight culture was centrifuged at 11500 rpm for 10 min at 25°C (Eppendorf centrifuge 5804 R) and supernatants were discarded and the pellets were collected before adding 150 µL of TE buffers and incubated at 37°C overnight. One µL of proteinase K (50 µg/µL Sigma) was mixed to 150 µL of Gram positive lyses solution and were then added to TE buffere mixtures and mixed well. The samples were incubated 65 between 79°C for 15 min and vortex every 5 min, used by placing in ice for 5 min. MPC protein precipitation reagent 175 µL were added to each sample then vortexed and centrifuged at 13000 rpm for 10 min at 4°C (Eppendorf centrifuge 5804 R). The supernatants were transferred in to new tubes and the pellets were discarded. One µL of RNase II (5 µg/µL) was added to each sample and mixed thoroughly. The samples were incubated at 37°C for 30 min and were added with 500 µL of iso-propanol to the supernatants and centrifuged at 13000 rpm (Eppendorf centrifuge 5804 R) at 4°C for 10 min. Isopropanol was removed using an eppendorf pipette without diloading the DNA pellet. The pellets were rinsed with 200 µL ethanol 70% and removed carefully and the DNA was resuspended with 35 µL of deionized water and was kept at -20°C for further analysis.

3.7.4 Polymerase Chain Reaction of lactic acid bacteria

Each sample of purified DNA was processed to the PCR using Fail Safe ™ Pre Mix Kit Epicentre® (An Illumina ® company), the oligonucleotide primer were used 16S forward (5'-AGAGTTTGATCCTGGCTC-3') and 16s reverse: (5'-CGGGAACGTATTCACCG-3') (Manusson et al., 2003). Primers were synthesized at 1st Base Malaysia. The setting of PCR was as follows: initial at 95°C for 2 min, de-naturation at 92°C for 45 s, annealing at 54°C for 1 min and extension at 72°C for 1 min, with 35 cycles for each steps. There were 2 µL of each amplification mixtures were subjected to electrophoresis in 1.5% (w/v) agarose gels in 0.5 × TAE buffer foe 45 min and 110 V. The DNA molecular mass marker (250 to 10000bp) molecular ladders from 1st Base, Malaysia was used as standard. After electrophoresis the gel was stained in ethidium bromide and after and was visualized and photographed with UV trans illuminator (BIORAD). The partial 16S rDNA sequences were determined by 1st Base, Malaysia and sequences were compared with databases (Gen-Bank).

3.8 Genotypic identification of fungi *Fusarium* species

3.8.1 Total genomic DNA extraction

Isolates fungi from severely infected different plant part were look like to the species *Fusarium* species on PDA medium based on mycelial plugs. However, fungi were determined by PCR Polymerase Chain Reaction using method Maja Ignjatov et al. (2012) with slightly modification.

The fungal isolates were grown in MEB medium and allowed to survive for 5 d at 28°C. After incubation the spores suspension and transferred to Eppendorf tubes for DNA extraction. Total genomic DNA of all fungi samples was extracted using Wizard ® Genomic DNA Purification Kit (Promega, USA) following the manufacturer's instruction. Fungal *Fusarium* species sample was obtained from well-grown MEB medium. The samples were suspended in 480 µL at 50mM EDTA. Next, 120 µL lyticase enzyme was added with the mixture before being incubated at 37°C for 60 min. The mixtures were then centrifuged for 2 min at 13,000 rpm and 600 µL nuclei lyses solution was added to the pellet obtained.

Subsequently, the mixture was mixed gently before incubating for 5 min at 80°C. Three µL of RNase solution was added to the mixture and was incubated at 37°C for 15 to 60 min. 200 µL of protein precipitation solution was added to the mixture, vortexed continuously and were then incubated in ice for 5 min. The mixture was then followed by centrifugation at 13,000 rpm for 3 min. The supernatant obtained was transferred to a clean tube containing 600 µL of room temperature (25°C) isopropanol and mixed properly. The mixture was centrifuged for another 2 min at 13,000 rpm, and the supernatant was discarded. After that, 600 µL of 70% ethanol was added, mixed and centrifuged again for 2 min at 13,000 rpm. The ethanol was aspirated and the pellet was air-dried for 10 to 15 min. The DNA pellet was rehydrated in 100 µL of rehydration solution for 1 h at 65°C or overnight at 4°C.

3.8.2 PCR amplification

Amplification was carried out using master mix (BIORON). The amount of mixture used is shown in Table 3 and 4. Polymerase chain reaction (PCR) amplification of internal transcribed spacer (ITS) DNA *Fusarium* samples were identified by sequencing the internal transcribed spacer (ITS) regions. To determine the identity of *Fusarium*, the amplified ITS PCR products obtained from total genomic DNA using primer set ITS1- primer pair: primer pair: Forward primer (ITS 1) (5′ATGGGTAAGGA (A/G) GACAAGAC-3′) and Reverse primer (ITS 4) (5′GGA (G/A) GTACCAGT (G/C) ATCATGTT -3′) (Geiser et al., 2004). The negative PCR non-template control (water only) and positive control (DNA extracted) from *Flammulina velutipes* is used as template.

TABLE 3: Polymerase chain reaction master mix

PCR Reaction Mixture	Amount (µL)
DNA template	2.0
Complete 10X PCR-Buffer (1 x)	5.0
dNTP mix (800 µM)	1.0
Forward primer (ITS 1) (0.5 µM)	2.5
Reverse primer (ITS 4) (0.5 µM)	2.5
DFS-Taq DNA polymerase (0.1 unit)	0.5
Sterile distilled water	36.5
Total volume of reaction	50.0

The amplification of PCR products was done by using T100 Thermal Cycler (Bio-Rad, Singapore). The PCR condition was stated in Table 4. The amplified DNA was kept at -20˚C until further analysis.

TABLE 4: PCR cycling condition for the therm-ocycler

Reaction Parameters	Temperature	Time
Initial Denaturation	95°C	5 min
(30 Cycles)		
Denaturation	95°C	1 min
Annealing	60°C	1 min
Extension	72°C	5 min
Final Extension	72°C	5 min
Hold	4°C	-

3.8.3 Gel electrophoresis

Gel electrophoresis was used to analyse the PCR product by separating the amplified DNA on agarose gel according to their size. The 1% (w/v) agarose gel was prepared by dissolving 0.4 g of agarose powder in 40 ml 1 × TAE buffer. Five µL of 1 kb ladder (Norgen High Ranger) was loaded into the well of agarose gel and used as a standard. Aliquots of 7 µL of amplified product were then loaded into the well together with 3 µL of loading dye (Norgen). The gel was run in TAE buffer at 100 V constant voltages for 35 min. The separated DNA was stained with gel red (Biotium) and was visualized using computer based gel documentation system (Uvitec Cambridge).

3.8.4 PCR products purification

PCR products were purified using high pure PCR product purification kit (Roche Diagnostic GmbH, Germany) by following the manufacturer's instruction. 5 volumes of binding buffer and 1 volume of PCR product was briefly mixed well and transferred into high pure filter tube. The DNA was bind to the filter by centrifugation at 13,400 rpm (Eppendorf mini spin, USA) for 60 sec at 5°C. The flow-through was then discarded. An additional 500 µL of wash buffer was added and centrifuged at 13,400 rpm for 1 min. The flow-through was also discarded. Additional centrifugation at 13,400 rpm for 1 min was performed after 200 µL of wash buffer was added. Finally, DNA was eluted into a clean 1.5 mL micro-centrifuge tube by adding 50 µL of elution buffer (10 mM Tris-HCl) and centrifuged at 13,400 rpm for 1 min.

3.8.5 PCR product sequencing

The DNA fragment of 5.8S-ITS was sent for sequencing to 1st base laboratory in Shah Alam, Selangor after PCR amplification using the ITS1 and ITS4 primers. The assembled sequences were aligned and compared with the GenBank databases of the National Center for Biotechnology Information (NCBI). Searches in GenBank with Basic Local Alignment Search Tool (BLAST) program were performed to determine the closest known relative of DNA fragment of 5.8S-ITS (Altschul et al., 1997). The sequences obtained were assembled using the Chromas Lite software, version 2.1.1.

3.9 Data analysis

Mean ± standard deviation obtained from each analysis was analyzed using One-way Analysis of Variance (ANOVA) mean and sigificant was done by the Tukey test at ($P<0.05$). The statistical analyses were performed using Minitab 16 software.

3.10 RESULTS

3.10.1 Isolates of Lactic acid bacteria

A total of 21 LAB isolates were characterized from 14 from at soil samples, 7 from fermented chilli, and 3 LAB strains IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014 and *Lb.* casei from ATCC culture collected from faculty of science and technology, Universiti Sains Islam Malaysia, (USIM) in Table 5.

TABLE 5: Characterization of lactic acid bacteria Catalase and Gram's stain tests

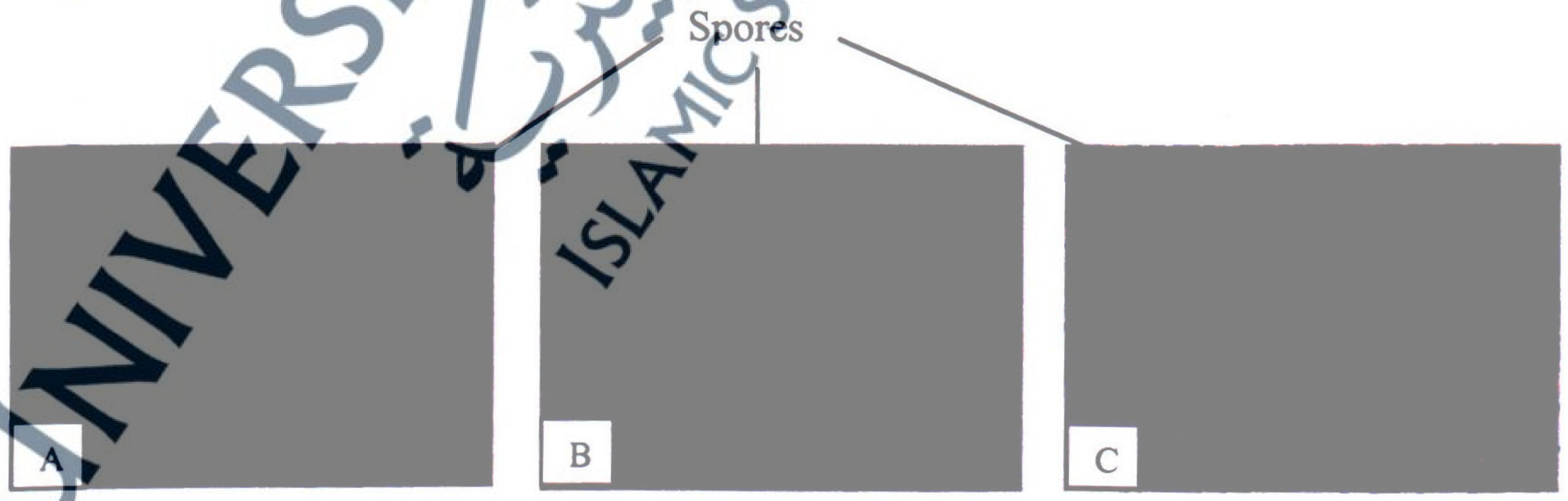
Isolates	Sources	Places	Catalase tests	Gram's stain test	Shapes
MSS1	Soil	Nilai land scape garden	Negative	Positive	Rod
MSS2	Soil	Nilai land scape Garden	Negative	Positive	Rod
MSS3	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS4	Soil	Subangjaya nursery	Negative	Positive	Cocci
MSS5	Soil	Nilai land scape Garden	Negative	Positive	Cocci
MSS6	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS7	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS8	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS9	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS10	Soil	Subangjaya nursery	Negative	Positive	Rod
MSS12	Soil	PuchongSelangor	Negative	Positive	Rod
MSS13	Soil	PuchongSelangor	Negative	Positive	Cocci

Continued					
MSS14	Soil	PuchongSelangor	Negative	Positive	Cocci
MSS15	Soil	PuchongSelangor	Negative	Positive	Cocci
FF11	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF16	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF17	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF18	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF19	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF20	Fermented chilli fruits	Local market	Negative	Positive	Rod
FCF21	Fermented chilli fruits	Local market	Negative	Positive	Rod

Notes:- MSS= isolates from soil, FF = from isolates fermented chilli fruit, and FCF = isoltes from fermented chilli fruits.

These isolates showed clear zone on the modified agar with 0.7% $CaCO_3$ and were catalase negative. Microscopic observation showed that the LAB isolates cocci and rod shapes which were isolated from soil and fermented chilli fruit as shown in Figure 2.

FIGURE 2: Isolation of Lactic acid bacteria (LAB) under the microscopic showing the shapes and size

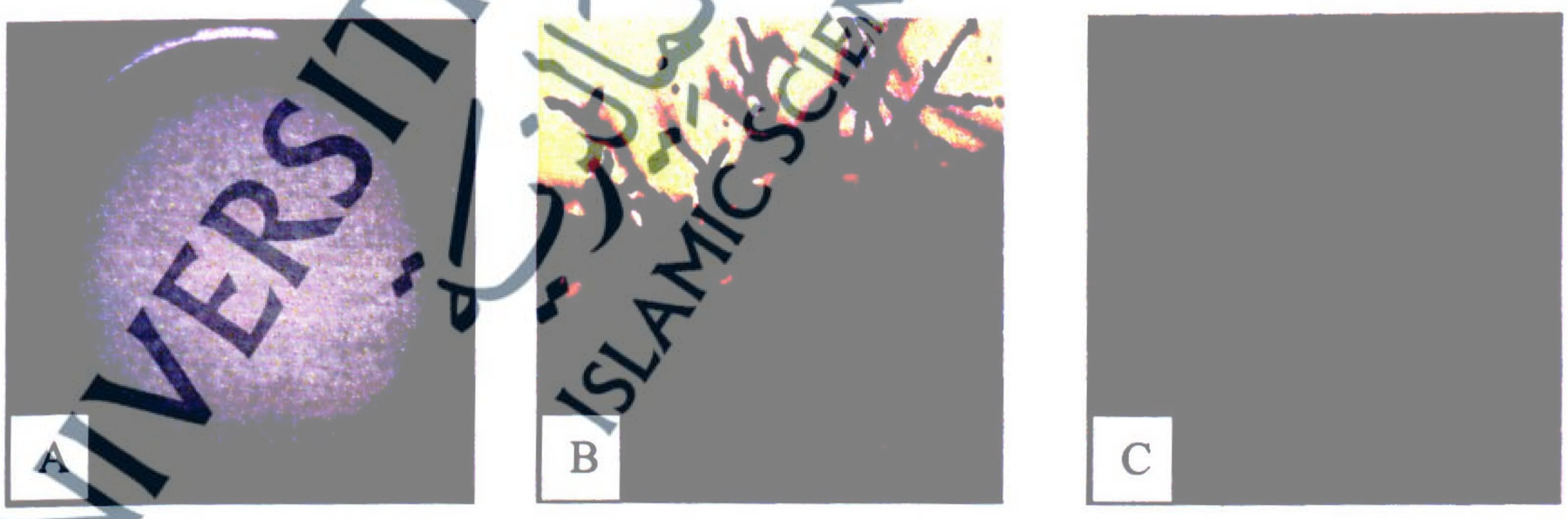


Notes:- A= cocci (LAB-MSS5), B= rod (LAB-MSS1) and C= rod (LAB-FF11)

3.10.2 Isolates of phytopathogenic fungi from different sources

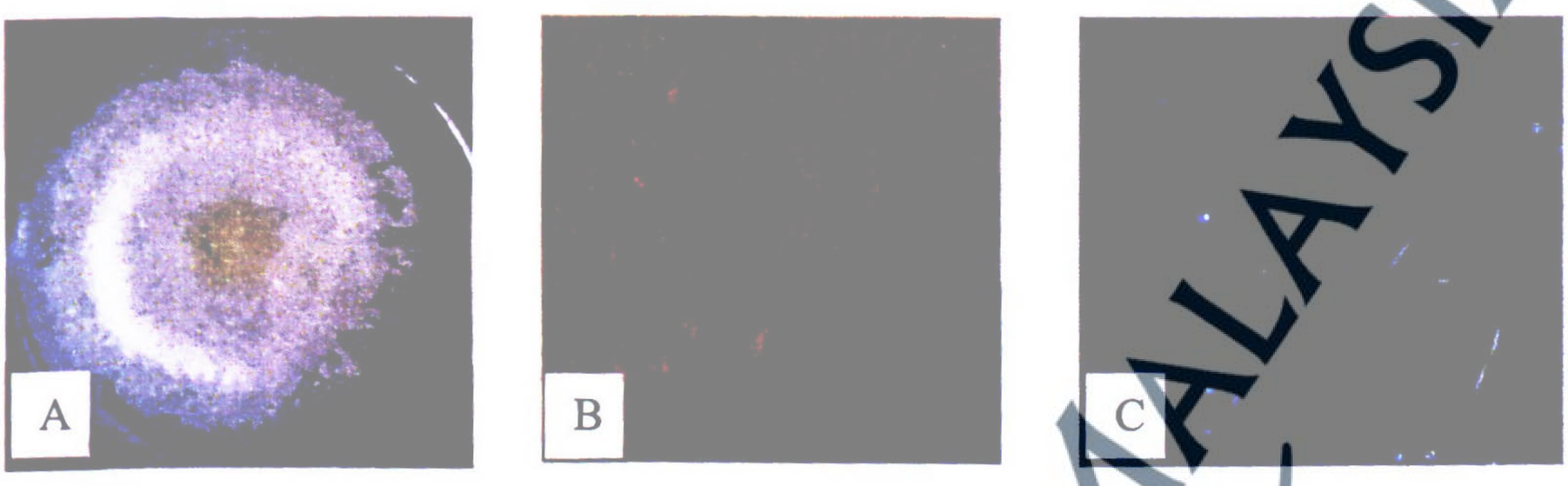
There are many samples of severely infected plants part were collected from Nilai Seramban Malaysia. The pure colony of fungal was obtained on PDA plate focused to mainly fungi *Fusarium* specie based on microscopic observation of morphology. Finally, with help of description different researchers for selection of *Fusarium* species. For instance, fungus isolate from chilli leaves (CL) was identified as *Fusarium oxysporum* f. sp. *lycopersici* following as morphological characteristics (Nirmaladevi1 and Srinivas 2012) as shown in (Figure 3). Fungal isolate from chilli seeds (CS) was identified as *Fusarium solani* similar to as described by Dhoro (2010) as shown in Figure 4., fungal isolate from chilli fruits (FC) as *Fusarium acuminatum* and fungal isolate from rose leaves LR as *Fusarium proliferatum* similar to the morphology as described by Yu (2010) as shown in Figure 5 and 6 and the all fungi isolates are listed in Table 6.

FIGURE 3: *Fusarium oxysporum* f. sp. *lycopersici* isolated from chilli leaves (CL)



Notes:- Morphology; A = culture on PDA, B = mycelia and C = spores

FIGURE 4: *Fusarium solani* isolated from chilli seeds (CS)

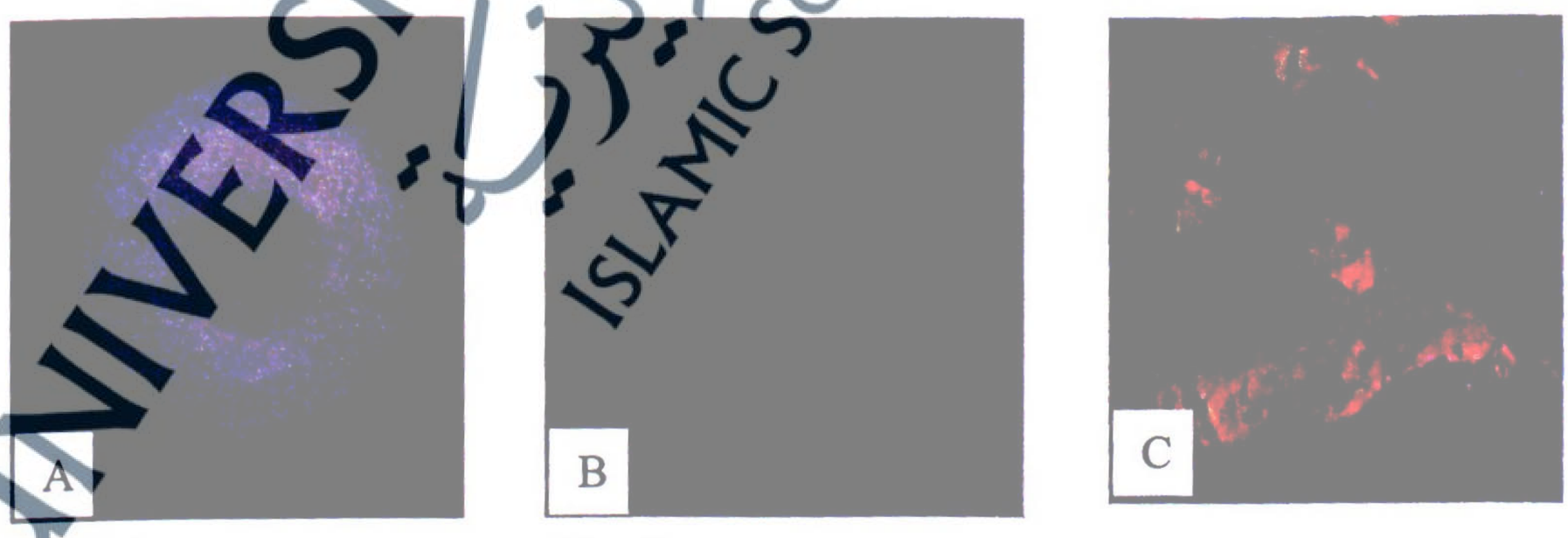


Notes:- Morphology; A = culture on PDA, B = mycelia and C = spores

FIGURE 5: *Fusarium acuminatum* isolated from chilli fruits (FC)

Notes:- Morphology; A = culture on PDA, B = mycelia and C = spores

FIGURE 6: *Fusarium proliferatum* isolated from rose leaves (LR)



Notes:- Morphology; A = culture on PDA, B = mycelia and C = spores

TABLE 6: Based on microscopic observation morphological characteristic of phytopathogenic fungi compared to previous researchers as *Fusarium* species

Fungi	Sources	References
CL	Chilli leaves	Nirmaladevi1 and Shrinivas, 2012
CS	Chilli seeds	Dhoro, 2010
FC	Chilli fruits	Yu, 2010
LR	Rose leaves	Yu, 2010

Notes:- CL = *Fusarium oxysporum* f. sp. *lycopersici*, CS = *Fusarium Solani*, FC = *Fusarium acuminatum* and LR = *Fusarium proliferatum*

3.10.3 Growth of phytopathogenic fungi using different media

The phytopathogenic fungi *Fusarium* species were easily grown in two types media namely MRSB and MEB, both media were observed that suitable for fungi *Fusarium* growth. However, the MEB was observed to be more suitable than MRSB as indicated by optical density in Table 7. Growth of fungi after 7 d incubation at room temperature (28°C) in MEB medium showed not significantly difference ($P>0.05$) increase in $OD630_{nm}$ range from 1.03 to 1.56. In contrast all the fungi grew well in MRSB with OD ranging significantly different ($P<0.05$) from 0.43 to 1.36 after seven days incubation.

TABLE 7: Growth of fungi in MRSB and MEB measured at OD630 nm

	MRSB		MEB	
	72 h	7 d	72 h	7 d
CL	0.34[b]	0.46[b]	0.83[a]	1.03[a]
CS	1.65[a]	1.36[a]	1.46[a]	1.55[a]
FC	0.34[b]	0.43[b]	0.86[a]	1.12[a]
LR	1.24[a]	1.33[a]	1.46[a]	1.56[a]

Notes:- Means with the same letter in the same column are not significant different (P>0.05) and Means with the different letter in the same column are significantly different; fungi were incubted for 72 h and 7 days at room temparature 28°C; CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

3.10.4 Growth of selected LAB in MRSB

The five LAB isolates were sellected based on inhibitory activity and, were grown in MRS broth especially LAB-FF11, LAB-MSS1 and IDLAB-6 with average absorbance at OD630 nm ranged from 1.48 to 1.68 (Table 8) did not show any significantly (P>0.05) difference. The greater growth optical densities using micro titer plate assay was recorded at 1.68 of LAB-FF11which isolated from fermented chilli fruit.

TABLE 8: Growth of LAB strains in MRS broth incubated at 37°C for 24 h

LAB strains	Sources	OD at 630 nm
LAB-MSS1	Soil	1.62[a]
LAB-MSS5	Soil	1.48[a]
IDLAB6	ATCC	1.60[a]
IDLAB7	ATCC	1.56[a]
LAB-FF11	Fermented chilli fruit	1.68[a]

Notes:- Means with the same letter in the same column are not significant different (P>0.05); LAB-MSS1 = *Lb. plantarum* from Malaysian soil sample, LAB-MSS5 = *P. pentoceous* from Malaysian soil sample, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, and LAB-FF11 = *Lb. plantarum* from fermented chilli fruits

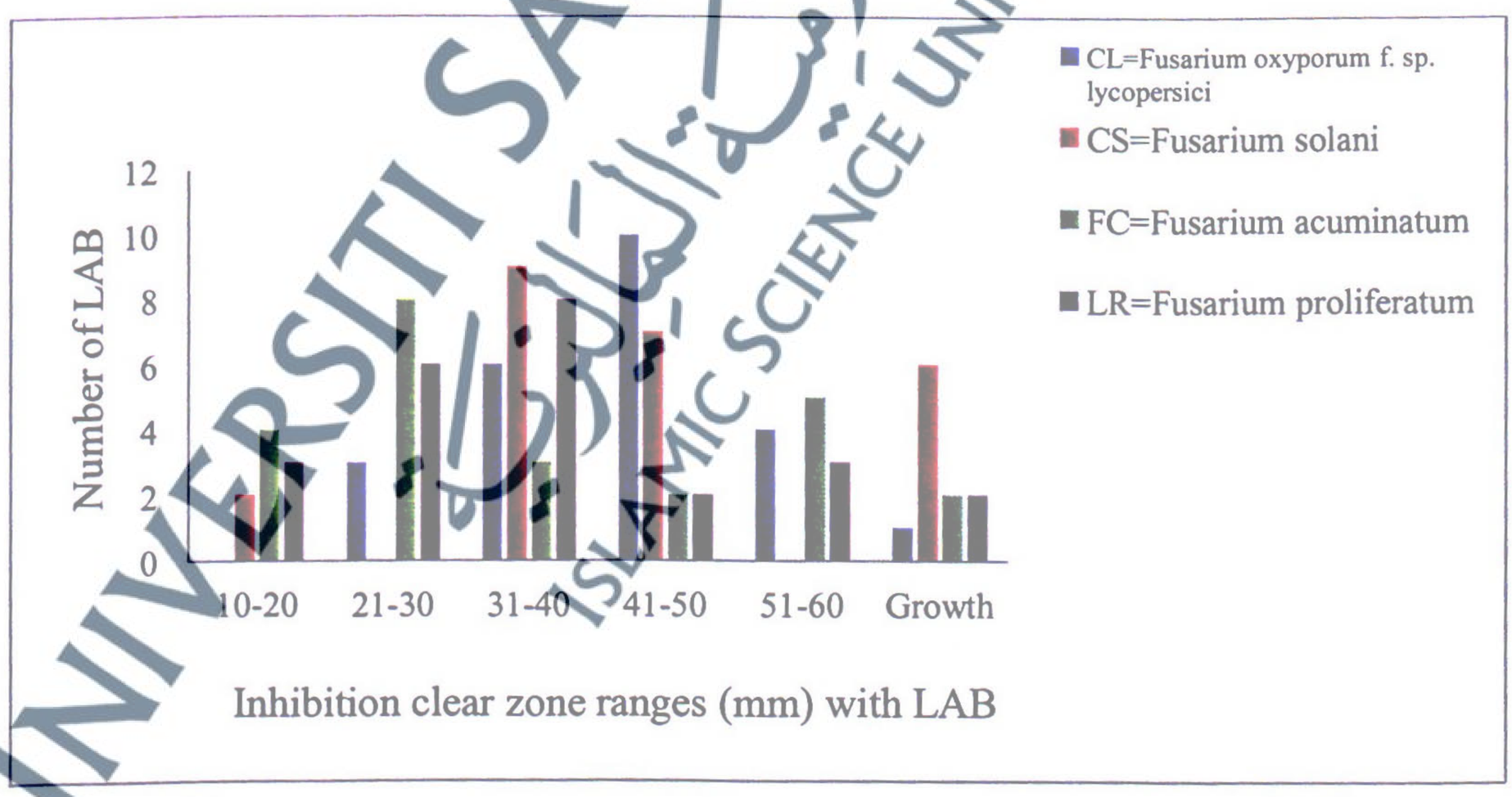
3.10.5 Qualitative biomass inhibition of *Fusarium* spp. in different method

3.10.5.1 Screening for antifungal activity of isolated LAB

The antifungal activity of isolates LAB against fungi was influenced by species of LAB and fungi. From 24 LAB isolates 19 showed good inhibitory activity against targeted phytopathogenic fungi *Fusarium* species using the dual overlay method (Figure 7 & 8). The inhibitory activity against *Fusarium* species varies with LAB isolates. *Fusarium oxysporum* f. sp. *lycopersici*-CL was readily inhibited by 20/24 of the LAB isolates with inhibitory zone 30 to 60 mm; the biggest inhibition was shown by LAB MSS5 within 48 h incubation. It was observed that inhibitory zone against *F.solani*-CS was variable where 7/24 isolates showed inhibitory zone of 40 to 50 mm, 9/24 isolates inhibited with 30 to 40

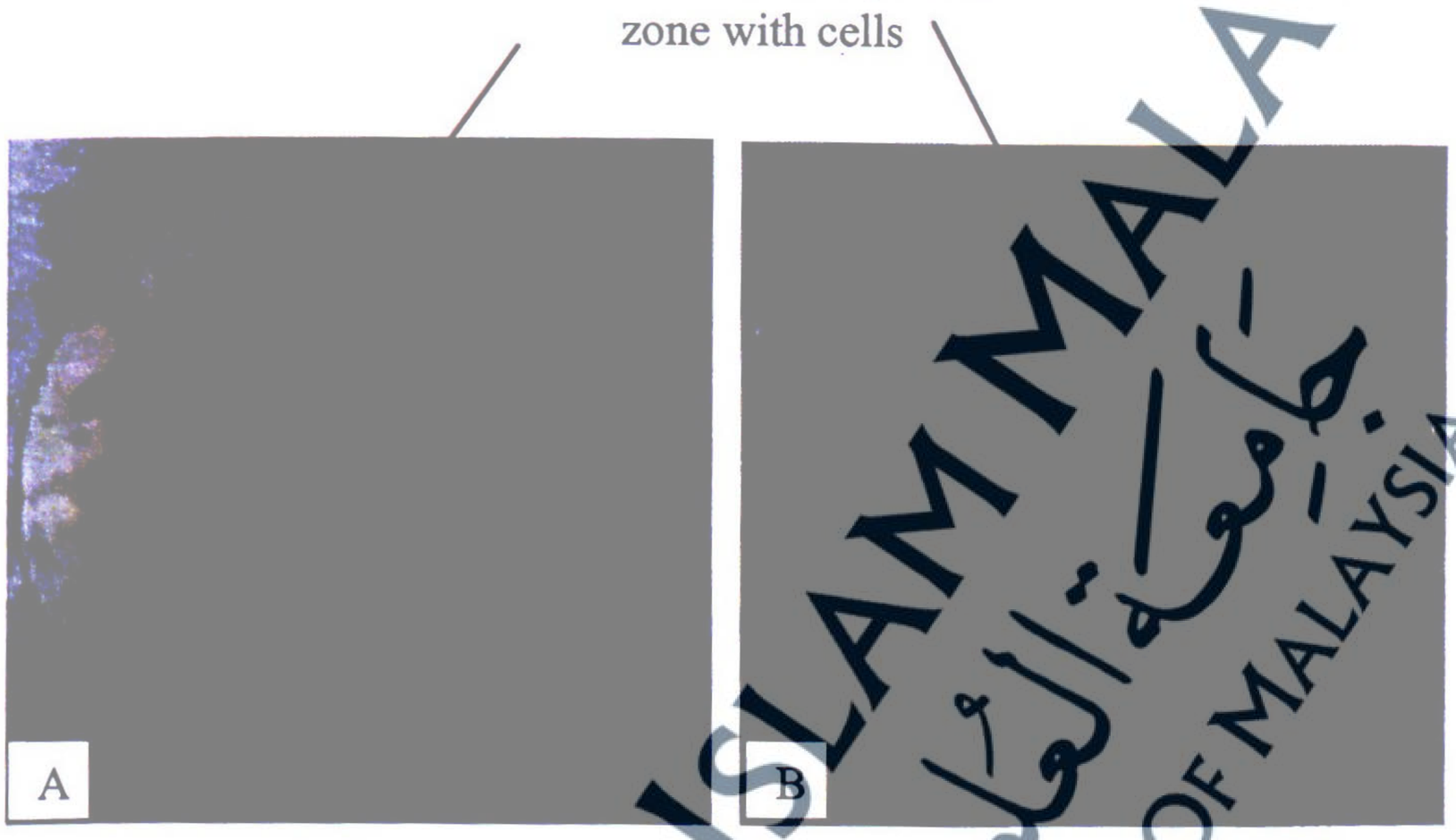
mm and six LAB isolates were not inhibitory. Fungi *F. acuminatum*-FC was more difficult to be inhibited by LAB cells; 10/24 LABs suppressed the fungal growth with inhibitory zone range between 30 to 60 mm. However, *F. proliferatum*-LR isolated from severely infected rose leaves was easily inhibited by all the LABs isolates with inhibitory zone from 10 to 60 mm. All the *Fusarium* species evaluated also 24, 48 and 72 h were showed good inhibitory activity. However these *Fusarium* species were not inhibited by LAB-MSS4 and LAB-MSS12 afer 48 h incubation at 28°C. Based on the results of the dual overlay method used for screening antifungal activity of LAB against *Fusarium* spp. five LAB strains were selected for further study.

FIGURE 7: Disribution of Lactic acid bacteria showing inhibitory activity (in mm) against *Fusarium* species by dual agar overlay method evaluated after 48 h incubation at 30°C



Notes:- Vertical = number of LAB inhibited *Fusarium* species and horizontal = range of inhibition clear zone *Fusarium* species by LAB isolates

FIGURE 8: Screening of LAB for antifungal activity against *Fusarium* species



Notes:- (A) = *F. oxysporum* f. sp. *lycopersici*-CL with LAB-MSS5 cells and (B) = *F. Acuminatum* with LAB-MSS1cells

3.10.5.2 Antifungal activity of LABs cell free Supernatants using well diffusion method

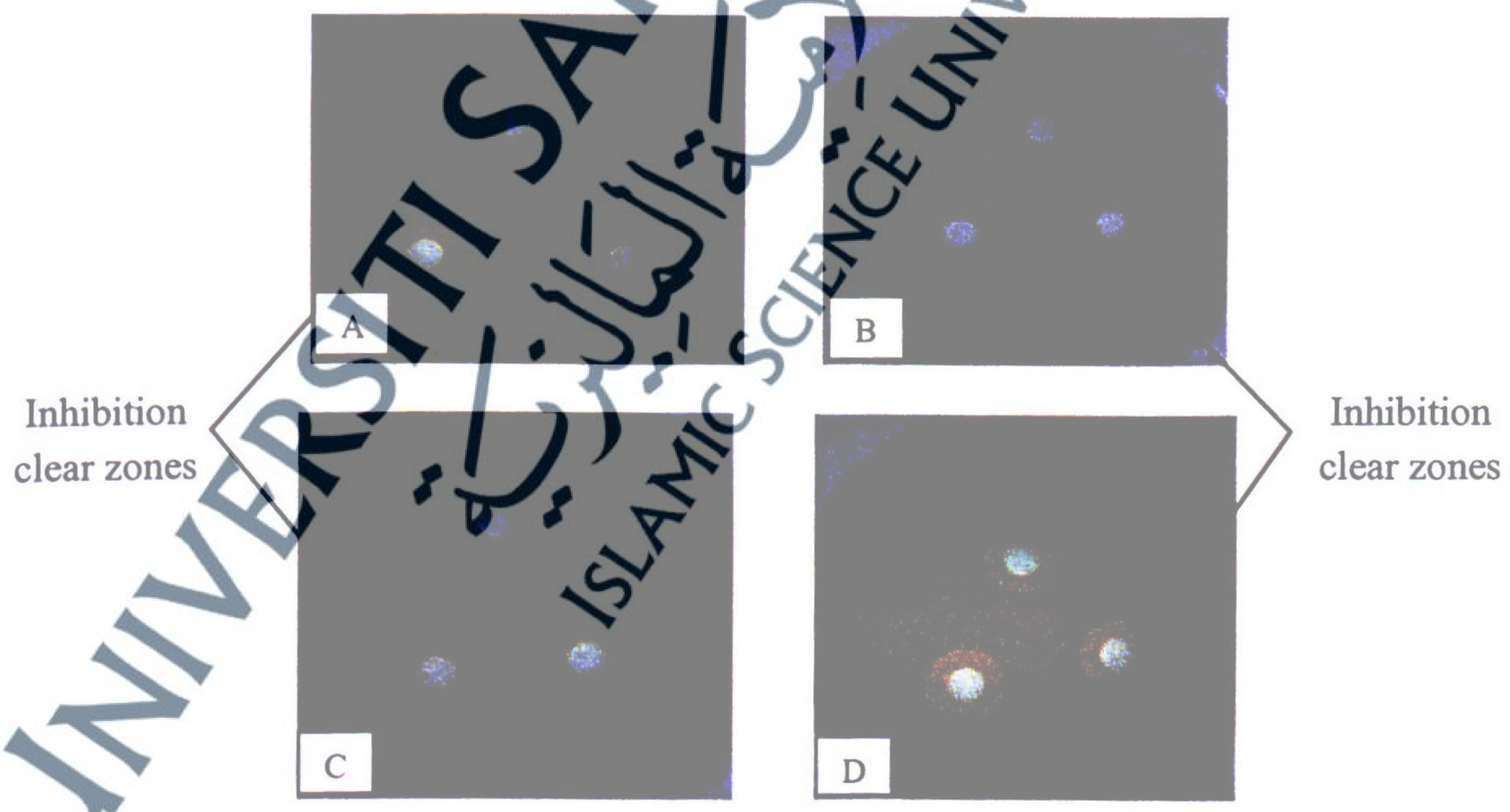
Well diffusion method was used to evaluate the inhibitory effect of the five selected LAB on conidia germination and the myceliagrowth of phytopathogenic fungi (Table 9 & Figure 9). It was observed that *F. solani*-CS was inhibited by all LABs with values ranged from 6.06 between 7.06 mm. However, *F. oxysporum* f. sp. *lycopersici*-CL, *F. proliferatum*-LR and *F. acuminatum*-FC were inhibited moderately by IDLAB6, IDLAB7 AND LAB-FF11 and the inhibition gowth range between 3.06 mm to 4.53 mm. However, *F. acuminatum*-FC was poorly inhibited by LAB-MSS1 with inhibitory zone at 2.76 mm after 48 h incubation.

TABLE 9: Antifungal activity of LAB-CFS evaluated by well diffusion method

LAB-CFS	Inhibition Mean± SD (mm)			
	CL	CS	FC	LR
LAB-MSS1	3.30^{b}	6.40^{ab}	2.76^{b}	3.76^{ab}
LAB-MSS5	3.23^{b}	6.06^{b}	4.53^{a}	3.26^{c}
IDLAB6	3.50^{ab}	6.53^{ab}	3.60^{ab}	3.93^{a}
IDLAB7	3.86^{a}	7.05^{a}	3.83^{ab}	3.60^{abc}
LAB-FF11	3.53^{ab}	7.06^{a}	3.06^{ab}	3.50^{bc}

Notes:- Means with the same letter in the same column are not significant different ($P>0.05$) and Means with the different letter in the same column are significantly different ($P<0.05$); LAB-MSS1 = *Lb. plantarum* from Malaysian soil sample, LAB-MSS5 = *P. pentoceous* from Malaysian soil sample, IDLAB6= *Lb. acidophilus* ATCC314,IDLAB7 = *Lb. plantarum* ATCC8014, and LAB-FF11 = *Lb. plantarum* from fermented chilli fruits and CL= *F. oxysporum* f. sp. *lycopersici,* CS = *F. solani,* FC = *F. acuminatum* and LR = *F. proliferatum*

FIGURE 9: Antifungal activity of LAB-CFS against different fungi as evaluated by well diffusion method



Notes:- Clear zoens showed fungal plug inhibitions; (A) = LAB-MSS5+CL, (B) = LAB-MSS1+CS, (C) = IDLAB6+FC and (D) = LAB-MSS5+LR

3.10.5.3 Antifingal activity supernatant of lactic acid bacteria on fungal growth using potato dextrose agar

The antifungal activity of LAB-CFS to inhibit *Fusarium* species mycelial growth using PDA method is showed in Table 10. The results found that the inhibitory activity was significantly different ($P<0.05$) against all *Fusarium* species after seven days incubation. It was observed that LAB-MSS5-CFS strongly reduced the spreading of the *F. Solani*-CS about 60.0% after 5 d incubation at room temparature 28°C.

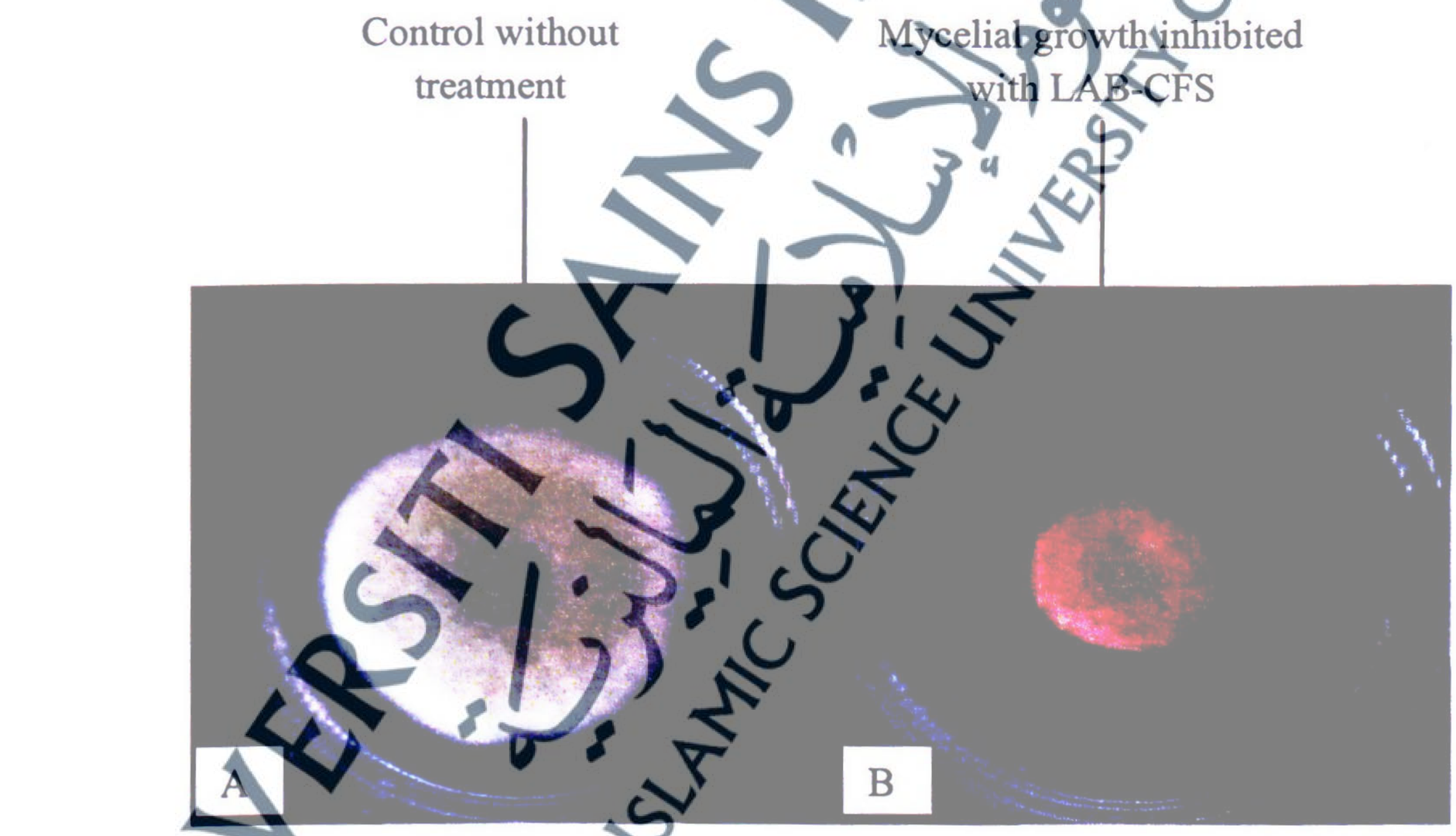
TABLE 10: Mycelium growth inhibition of *Fusarium* species using LAB-CFS evaluated on PDA after 7 d incubated at room temperature 28°C

LAB-CFS	Inhibition (%)			
	CL	CS	FC	LR
LAB-MSS1	24.3^{c}	35.0^{e}	47.8^{c}	32.9^{e}
LAB-MSS5	31.8^{a}	60.0^{a}	36.7^{e}	35.6^{d}
IDLAB6	23.5^{b}	40.0^{b}	65.0^{a}	36.1^{c}
IDLAB7	24.2^{b}	38.0^{c}	44.4^{d}	35.6^{b}
LAB-FF11	20.4^{e}	36.0^{d}	55.5^{b}	43.8^{a}

Notes:- Means with the same letter in the same column are not significant different ($P>0.05$) and Means with the different letter in the same column are significantly different ($P<0.05$); LAB-MSS5 = *P. pentoceous* from Malaysian soil sample, IDLAB6 = *Lb. acidophilus* ATCC314, IDLAB7 = *Lb. plantarum* ATCC8014, and LAB-FF11 = *Lb. plantarum* from fermented chilli fruits and CL = *F. oxyporum*f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

However, the percentage mycelial growth of *F. oxysporum* f. sp. *lycopersici*-CL was stable percentage 35.0% because growth was supressed by LAB-MSS1-CFS. The *Lb. acidophillus* ATCC314 inhibited mycelia spread of *F. acuminatum*-FC by 65.0% in Figure 10. It was observed that LAB-MSS1-CFS and LAB-FF11-CFS were also suppressed the colony growth of *F. proliferatum*-LR from 32.9% to 43.8% after incubation 7 days.

FIGURE 10: Mycelium growth inhibition of *Fusarium* species using LAB-CFS observed on PDA after 7 d at room temperature (28°C)



A= *F. acuminatum*-FC

B= LAB-MSS1 CFS+FC

3.10.6 Quantitative biomass inhibition of *Fusarium* species in different media

3.10.6.1 Biomass inhibition in MRSB media with LAB cells

The growth of fungi in MRSB with added LAB cells reduced fungal mass variable and did not show significant ($P<0.05$) inhibitory activity of *Fusarium* species. It was observed that germination of inoculated conidial spores of target fungi to MRSB was inhibited by addition of LAB cells as shown by percentage of inhibition in mycelial mass ranged from 76.9 to 96.1% for all the fungi (Table 11).

TABLE 11: Percentage biomass reduction of fungi *Fusarium* species using cells of LABs in MRSB after incubation for 7 d at room temperature 28°C

LABs-Cells	Mycelia mass reduction (%)			
	CL	CS	FC	LR
LAB-MSS1	93.33[a]	91.07[a]	90.37[a]	79.93[a]
LAB-MSS5	87.16[a]	97.43[a]	82.72[a]	76.93[a]
IDLAB6	91.39[a]	91.96[a]	87.43[a]	92.32[a]
IDLAB7	96.61[a]	85.72[a]	78.55[a]	82.92[a]
LAB-FF11	96.09[a]	94.32[a]	90.90[a]	87.59[a]

Notes:- Means with the same letter in the same column are not significant different ($P>0.05$); LAB-MSS1= *Lb. plantarum* from Malaysian soil sample, LAB-MSS5= *P. pentoceous* from Malaysian soil sample, IDLAB6 = *Lb. acidophilus* ATCC314,IDLAB7 = *Lb. plantarum* ATCC8014, and LAB-FF11 = *Lb. plantarum* from fermented chilli fruits and CL = *F. oxysporum* f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatum*

However, the antifungal activity of LAB cells against *Fusarium* species varied evaluated. The LAB-MSS5 inhibitory activity against growth of *F. solani*-CS by 97.4% but less for *F. proliferatum*-LR about 76.9%. It was also observed that cells of IDLAB7 reduced the growth of *F. oxysporum* f. sp. *lycopersici*-CL by 96.6% but growth of *F. acuminatum*-FC was reduced by 78.5%. Similarly, LAB –FF11 was effective to reduce growth of *F. acuminatum*-FC ranged from 90.9% to 97.6 % for all the fungi. The cells of all LABs were recorded to inhibit the *Fusarium* species greater than MEB because, MESB was suitable medium for LAB growth.

3.10.6.2 Biomass inhibition in MEB media with LABs supernatants

The growth of fungi in MEB with added LAB CFS reduced fungal mass variable and showed no significant inhibitory activity of *F. oxysporum* f. sp. *lycopersici*-CL, *F. solani*-CS and *F. acuminatum*-FC. However, the growth of *F. proliferatum*-LR was inhibited significantly after incubation for 7 day at room temperature 28°C. The better inhibition effect was observed against *F. solani*-CS, it was highly inhibited (91.8%) by IDLAB6-CFS strain. The lowest biomass inhibition of *F. proliferatum*-LR was observed by LAB-MSS5-CFS about 29.9%. However, *F. acuminatum*-FC and *F. oxyporum* f. sp. *lycopersici*-CL were moderately inhibited about 63.2%, and 68.7 % by CFS of LAB-MSS1 in (Table 12) respectively.

TABLE 12: The percentage biomass reduction of fungi *Fusarium* species by LAB-CFS after incubation for 7 d at room temperature 28°C

LAB-CFS	Mycelia mass reduction (%)			
	CL	CS	FC	LR
LAB-MSS1	68.74[a]	71.41[a]	63.23[a]	34.08[b]
LAB-MSS5	78.72[a]	73.80[a]	57.38[a]	29.92[b]
IDLAB6	75.62[a]	91.8[a]	59.53[a]	59.19[a]
IDLAB7	75.62[a]	86.86[a]	57.47[a]	62.19[a]
LAB-FF11	80.00[a]	80.03[a]	69.72[a]	56.36[a]

Notes:- Means with the same letter in the same column are not significant different ($P>0.05$) and Means with the different letter in the same column are significantly different ($P<0.05$); LAB-MSS1=*Lb. plantarum* from Malaysian soil sample, LAB-MSS5 = *P. pentoceous* from Malaysian soil sample, IDLAB6 = *Lb. acidophilus* ATCC314,IDLAB7 = *Lb. plantarum* ATCC8014, and LAB-FF11 = *Lb. plantarum* from fermented chilli fruits and CL = *F. oxyporum*f. sp. *lycopersici*, CS = *F. solani*, FC = *F. acuminatum* and LR = *F. proliferatu*

3.11 Identification of LAB Isolates

3.11.1 Phenotypic identification using API 50 CH kit

Phenotypic identification of six LAB isolates from soil and one from fermented chili fruit samples using API 50 CH kit showed that the isolates MSS1, MSS2 and FF11 was 99 % similarity with *L. plantarum*1 and isolates MSS5 showed 82.00% similarity with *P. pentosaceus*1. However, MSS13, MSS14 and MSS15 were 48.4% similarity with *L. lactiss sp. lactis* 1 and isolates of LAB identify using API 50CH kit assay (Table 13) and appendix-A-Figure 46 A & B.

TABLE 13: Identification of LAB isolated Malaysian soil samples and fermented food by API 50CHL Kit.

Isolates	API 50CHL		
	Sources	Similarity	Identification
MSS1	soil	99.9%	*Lb. plantarum*1
MSS2	soil	99.9%	*Lb. plantarum*1
MSS5	soil	82.0%	*P. pentosaceus*1
MSS13	soil	48.4%	*Lb. lactis*ssp.*lactis* 1
MSS14	soil	48.4%	*Lb. lactis*ssp.*lactis* 1
MSS15	soil	48.4%	*Lb. lactis*ssp.*lactis* 1
FF11	Fermented chilli fruit	99.9%	*Lb. plantarum*1

Notes:- MSS = isolates from soil, FF = isolates fermented chilli fruits and FCF= isolted from fermented chllili fruits

3.11.2 Genotypic Identification using 16s r-DNA

Genotypic identification of two LAB isolates from soil and one from fermented chili fruit samples using 16s r-DNA showed that the isolates MSS1, FF11 were 100% similarity with *Lb. plantarum* and MSS5 99.00% were similarity with *P. pentoceous* (Table 14 and Figure 11).

TABLE 14: Identification of antifunal strains isolated Malaysian soil samples (MSS) and fermented food (FF) by 16S rDNA

Isolates	16S rDNA		
	Similarity	Identification	Accession
MSS1	100%	*Lb. plantarum*	KM207826.1
MSS5	99.00%	*P. pentoceous*	KP189228.1
FF11	100%	*Lb. plantarum*	CP010528.1

Notes:- MSS1, MSS5= isolates from soil and FF11= isolates fermented chilli fruits

FIGURE 11: Identification of LAB by PCR and the DNA bands on gel electrophoresis using 16S forward: and 16S reverse

Notes:- Showing LAB isolates; (1) *Lb. plantarum*, (2) *P. pentoceous*, (3) *Lb. plantarum* and (M) marker

3.12 Identification of *Fusarium* species using internal transcribed spacer

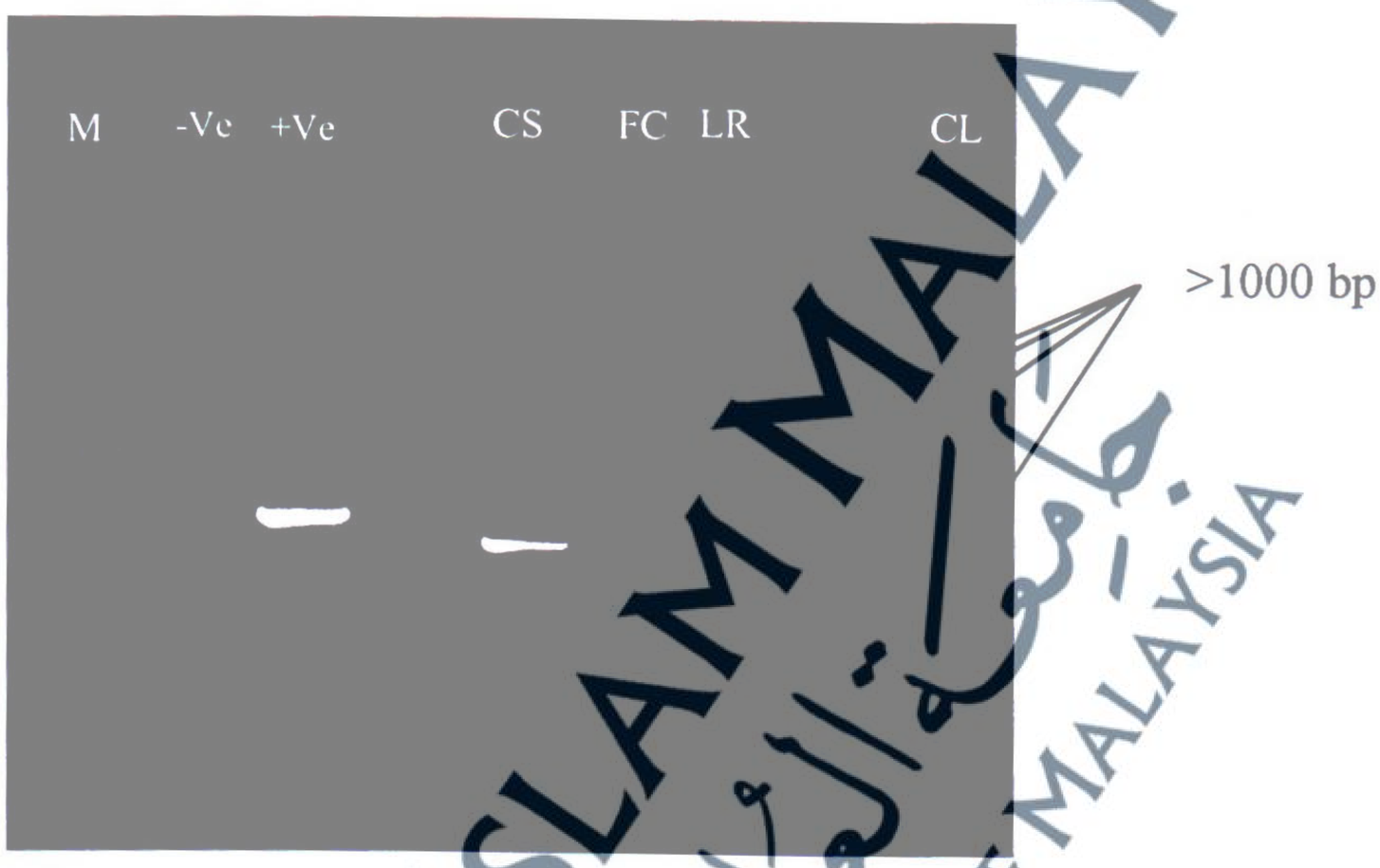
Identification of four fungi isolates from different plant parts using internal transcribed spacer (ITS) method showed that the isolates from chilli leaves (CL) and rose leaves were (LR) *Fusarium oxysporum* strain KAML01 and *Fusarium proliferatum* with was 99% similarity, respectively. However, the fungi from chilli seeds (CS) and chilli fruits (FC) were identified as *Fusarium* sp. CID124 and *Fusarium* sp. fus 124 with 99 % similarity, respectively as shown in Table 15. The size of DNA base pair was estimated 1000 KDa for *Fusraium* species such as *F. oxysporum* strain KAML01, *F. proliferatum, Fusarium* sp. CID124 and *Fusarium* sp. fus 124 using DNA extraction kit. However, the gel electrophoresis of DNA base pair *Fusarium* sp. CID124 was stronger in appearance compared to *F. oxysporum* strain KAML01, *Fusarium* sp. fus 124 and *F. proliferatum* isolates (Figure 12).

TABLE 15: *Fusarium* species identified by internal transcribed spacer

Fungi Isolates	Internal transcribed spacer (ITS) identification			
	Sources	Similarity	Identification	Accession
CL	Chilli leaves	99%	*F. oxysporum* KAML01	KC119203.1
CS	Chilli seeds	99%	*Fusarium* sp. CID124	EF589878.1
FC	Chilli fruits	99%	*Fusarium* sp. fus 124	EF589878.1
LR	Rose leaves	99%	*F. proliferatum*	FJ040179.1

Notes:- Sources of Fungi isolates; CL = chilli leaves, CS = chilli seeds, FC = chilli fruits and LR = leaves of rose plant

FIGURE 12: *Fusarium* species identification using PCR and the bands on gel electrophoresis



Notes: -ve: PCR non-template control (water only) +ve: Positive control (DNA extracted from *Flammulina velutipes* is used as template)

3.13 DISCUSSION

Most of the study on LAB conducted from food sources (fermented or raw) but, this study isolated 14 LAB strains from soil and rhizospheric soil samples in Malaysia as shown in (Table 5). The soil LAB showed antifungal activity against *Fusarium* spp. were identified as *Lb. plantarum*1 with 99.9% similarity for isolate MSS1 and MSS5, *Pediococcus pentosaceus*1 with similarity index of 82% for isolate MSS5. *Lb. lactis* sp. *lactis*1was also identified for isolates MSS13, MSS14 and MSS15 but the similarity index was low 48.4% as listed in (Table 13). Similarly, Chen et al. (2005) isolated LAB from different plants rhizospheric soil and identified them as *Lactococcus lactis* sp. *lactis, Lb. plantarum, Lb. paracasei* ssp. *paracasei*, *Lb. brevis, Leuconostoc mesenteroides* sp.

mesenteroides, Leuconostoc fallax, Weissella paramesen teroides and *Enterococcus* sp. *Fusarium* spp. such as *F. oxysporum*-1 *R. solani*-1 *F. oxysporum-2 R. solani-2 S. rolfsii* were high virulent strains of pathogenic fungi that often infected plants (Hoda et al., 2011) thus, the LAB isolates could be used as biocontrol agent against pathogenic fungi.

Therefore in this study out of 13 of 14 LAB isolated from soil showed good inhibitory against *Fusarium oxysporum f.* sp. *Lycopersici*-CL; *F. solani*-CS; *F. acuminatum*-FC and *F. proliferatum*-LR. However, five isolates which were LAB MSS10, LAB-MSS11, LAB-MSS12, LAB-MSS13, LAB-MSS14, and LAB-MSS15 did not showed inhibitory activity against *F. solani*-CS indicating specifity of LAB to target phytofungi (Figure 7). Haggag, (2008) reported that *F. solani* and *R. solani* causes severe damage to tomato cultivars in Egypt and, these pathogenic fungi were found resistant to both biocontrol agents and fungicides. Certain species of *Fusarium* such as *F. solani* sp. complex (FSSC) and *Fusarium keratitis* were reported by Centres for Disease Control, USA to cause outbreak of sight-threatening of corneal infections (Chang et al., 2006). The seven LAB from fermented fruits sources and three LAB from ATCC cultures were also tested for their inhibitory activity against phytopathogenic fungi *Fusarium* species. The LAB inhibited the growth of all the targeted pathogenic fungi *F. oxysporum* f. sp. *lycopersici*-CL, *F. solani*-CS. *F. acuminatum*-FC and *F. proliferatum*-LR and these targeted pathogenic fungi were isolated from different plants parts details morphology and characteristics as shown in (Figure 3,4, 5 & 6 and in Table 6). Baed on genotypic identification fungi Fusarium specis were identified *Fusarium oxysporum* strain KAML01, *F. proliferatum, Fusarium* sp. CID124 and *Fusarium* sp. fus 124 isolates were

shown in Table 15. Similarly, Yousef and Lloyd (2008) reported that *Lb. paracasei* sp. *Tolerans* completely inhibited the growth of *F. proliferatum* M 5689, M 5991and *F. graminearum* R 4053 compared to controls in a dual agar plate assay. It was observed that both cells and CFS of two soil borne (LAB-MSS1 and LAB-MSS5), one fermented chilli fruits (LAB-FF11) and two ATCC cultures (IDLAB6 and IDLAB7) suppressed the growth of *F. oxysporum* f. sp. *lycopersici*-CL; *F. solani*-CS; *F. acuminatum*-FC and *F. proliferatum*-LR as evaluated by well diffusion method and on PDA medium. The supernatants of LAB-FF11 inhibited *F. solani*-CS (Table 11 & Figure 9) whereas LAB-MSS5-CFS strongly reduced the spreading of the *F. Solani* CS (Table 9 & 9).

Additionally many researchers used the LAB-CFS to inhibit mycelia growth on fungi but no information available to inhibit the fungi with cells of LAB strains. In this study 18 h old LAB cells were used to inhibit the mycelia growth of fungi. Thus, are differences in biomass inhibition of *Fusarium* spp. grown in MRSB with added LAB cells was observed. Greater mycelia reduction was observed in the growth of *F. solani*-CS with LAB-MSS5 cells in MRSB about 97.4% (Table 11). However, growth of *F. proliferatum*-LR inhibition was not much better than other fungi but IDLAB6 showed better inhibition about 92.3% of mycelia growth of same *Fusarium* sp. Furthermore, MRSB is a good media for the growth of LAB strains (Table 8). It was expected that the metabolites produced by the LAB in MRSB could affect directly on the mycelia growth of the fungi.

The variability in cell mass reduction could be related to specificity of metabolites produced by LAB that may have caused failure of conidia germination and mycelium proliferation. Similar observation was noted by Muhaildin et al. (2011) reported that cell

mass of *A. oryzae,* a food spoilage fungi was inhibited by LAB-CFS Te010 and G004 in the liquid system. The antifungal activity of CFS of LAB against *Fusarium* spp. was further challenge in a MEB media that readily support the growth of fungi. In contrast to *F. solani*-CS was inhibited by IDLAB6 CFS. Evaluating the antifungal activity of LAB-CFS isolated from different sources and reported that cell mass of high virulent strains of pathogenic fungi *F. oxysporum* f. sp. *lycopersici, F. solani, F. acuminatum* and *F. proliferatum* that were isolated from diseased plants reduced when grown in MEB medium.

The cell mass was reduced by this study the cell mass was reduced 91.8% compare to control as described in (Table 12). However, all the LAB-CFS evaluated in this study reduced cell mass by greater than that reported by Hoda et al. (2011). Additionally, the phytopathogenic fungi *C. gloesporioides* and *C. capsici* were strongly inhibited by CFS of *Lb. plantarum*LAB-C5 and LAB-G7 isolated from Malaysian fruits (El-Mabrouk et al., 2012). The differences in antifungal activity of LAB CFS may related to the amount of the antifungal metabolites produced by the LAB strains, the specificity of the metabolites to fungal structure, growth density of fungi and hardness of mycelia.

3.14 CONCLUSION

LAB from soil samples inhibited the growth of *Fusarium* spp. especially *Fusarium solani*-CS which is very virulent pathogenic fungi for chilli plants better that LAB isolated from foods and ATCC cultures. Both LAB cells and supernatants showed strong

inhibitory activity against *Fusarium* species, whereby the cells of selected LABs when growth together with the fungi was more suitable than supernatants to suppress the biomass growth of all four *Fusarium* isolates studied. Therefore, the antifungal activity of LAB isolates could be showed from consists of protieneous compound in LAB thus, sensitivity of consistent of protieneous compound determined in next chapter.