© 2017 IJSRSET | Volume 3 | Issue 5 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
Themed Section: Science and Technology

Factors Effect on Antifungal Activity of Lactic Acid Bacteria against *Fusarium Proliferatum* Isolate from Rose Leaves

Akaram Husain[*1], Zaiton Hassan[1], and Mohd Nizam Lani[2]

[*1]Food Biotechnology Department, Faculty of Science and Technology, University Sains Islam Malaysia, Bandar Baru Nilai, Negeri Sembilan, Malaysia

[2]School of Food Science and Technology, University Malaysia Terengganu, Kuala Terengganu, Malaysia

ABSTRACT

Microorganism's lactic acid bacteria (LAB) are known to have antifungal activity, but its utilisation, as protection agent against fungi in plant is limited. The present study aimed at evaluating lactic acid bacteria (LAB) as bio-control agent against fungi *F. proliferatum* isolated from rose leaves. Five LAB strains namely, *Lactobacillus plantarum* 1MSS and *Pediococcus pentosaceus* 1MSS, *Lactobacillus plantarum* 1FF, *Lactobacillus acidophilus* ATCC 314 and *Lactobacillus plantarum* ATCC 8014 were evaluated for antifungal activity against the *F. proliferatum*-LR by several methods. Cells free supernatants (LAB-CFS) of all isolates showed strong antifungal activity (3.26 mm to 3.76 mm) evaluated by well diffusion method (43.00 mm to 49.66 mm) within 48 h at 28^{o}C. LAB-CFS reduced fungi hyphal growth on potato dextrose agar (PDA) and in malt extracts broth (MEB) from 32.9% to 43.8% and 93.79% to 94.65%, respectively. Enzyme treatments using pepsin, papain and proteinase K reduced the antifungal activity from 63.85% to 87.21% depending on enzyme used and LAB-CFS. Adjusting pH of LAB-CFS (pH 2 to 5) did not diminish the antifungal activity of *Lb. plantarum* 1MSS and *P. pentosaceus* 1MSS. The antifungal activity of cell supernatant of LAB was maintained after subjected to 80^{o}C and 90^{o}C and 121^{o}C for 30 min. Growth of *F. proliferatum* was inhibited by both cells and supernatants of the LAB. The LAB-CFS contains heat stable compounds that effective to be used as bio-control against *F. proliferatum*-LR that commonly infect rose plants.

Keywords: *Fusarium proliferatum*-LR, Lactic Acid Bacteria, Antifungal Activity.

I. INTRODUCTION

The most important pathogens that cause by *Fusarium* species are pathogenic to plants ([1- 3] and recognized for causing serious plant diseases on a number of economically and commercially important plants worldwide, including in Malaysia [4]. *Fusarium verticillioides* and *F. temperatum* and *F. subglutinans* cause large crop damage such as root, stalk and ear rot diseases [5]. The fungi have ability to produce a variety of fungal toxins, mainly zearalenols, zearalenone, trichothecenes, fumonisins, moniliformin and fusarins which threaten the health of humans and animals that consumes them [6]. Food and Agriculture Organization (FAO) of the United Nation.

Approximated more than 25% food crops in the world are vanished yearly because of mycotoxin contamination with the *Fusarium* species [7- 10]. The removal of fungal contamination is usually by heat or chemical treatment. It is essential to replace chemical fungicides to avoid pollution and health problems, thus alternative antifungal agents produced by microorganisms may be used as bio-control agent against pathogenic fungi [11]. Lactic acid bacteria (LAB) produced compounds that have ability to reduce the biomass and growth of *Fusarium* species [12]. Isolates *Lb. plantarum* LB20 and *Lb. plantarum* LB54 have antifungal activity against *Aspergillus* spp., produced heat stable compounds and active in acidic pH [13]. Many research reported antifungal activity of LAB from different sources, but few reports on LAB strains from natural soil. Therefore, this study evaluated the antifungal activity of LAB isolated from rhizospheric soil and fermented chilli fruits against *F. proliferatum*-LR isolated from severely infected ornamental plant rose leaves.

II. METHODS AND MATERIAL

A. Preparation of Cells Free Supernatant from Lactic Acid Bacteria

Phenotypically and genotypically identified LAB isolates *Lactobacillus plantarum* 1MSS and *Pediococcus pentosaceus* 1MSS, *Lb. plantarum* 1FF, *Lb. acidophilus* ATCC 314 and *Lb. plantarum* ATCC 8014 are used and identification and characteristics have been described in previous study [14] . The LAB isolates were inoculated into MRS broth (Oxoid, CM0359) and incubated for 24 h at 37°C in aerobic shaker incubator (SASTEC Laboratory Equipment, Malaysia) using method described by [15]. 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 were filtrated using sterile filter 0.45 µm-pore-size Millipore filter (Schleicher & Schuell, Dass El, Germany).

B. Determination of Inhibitory Activity on Fungal Spore Germination by Well Diffusion Method

The *Lactobacillus plantarum* 1MSS and *P. pentosaceus* 1MSS, *Lb. plantarum* 1FF, *Lb. acidophilus* ATCC 314 and *Lb. plantarum* ATCC 8014 isolates that showed strong activity were further tested for their anti-spore germination activity using the well diffusion method [16] with modification. In this method genotypically identified *F. proliferatum*-LR was targeted further observation *and* morphological characteristics and identification has been described in previous study [14]. One mL spore suspension (10^5/ml) from five days old fungi grown in malt extract broth (MEB) were spread plated on malt extract agar (MEA) and allowed to dry in laminar flow. Then, wells of size 7 mm were made using flame sterilized cork borer and 1-2 drops MEA agar was pipetted to cover the base of the well to avoid leaking of the supernatants. A 200 µL filtered of LAB supernatant were added to each well and the plates were incubated at 28°C for 48 h. The diameter of mycelia growth inhibition zone was measured in millimetre.

C. Evaluation of Antifungal Activity on Potato Dextrose Agar

Antifungal activity of cells free supernatants (CSF) of *Lb. plantarum* 1MSS and *P. pentosaceus* 1MSS, *Lb. plantarum* 1FF, *Lb. acidophilus* ATCC 314 and *Lb. plantarum* ATCC 8014 against *F. proliferatum*-LR on potato dextrose agar (PDA). Iolates LAB supernatants were filtered using 0.45 µm-pore-size filter Millipore (Schleicher & Schuell, Dassel, Germany) and the CFS of LAB were tested against fungi by mixing 1 ml of supernatant with 100 ml PDA (OXOID CM0139) and poured into petri dishes. Fungal mycelia were carefully placed in the centre of the Petri dishes and incubated at room temperature for 5 days [17] with modifications. The diameter of fungal growth was recorded. The percentage inhibition of mycelia growth was calculated using the formula GI= [(TC-TT)/TC] x100; where GI refers to growth inhibitions (%), TC (%) = total fungal growth on PDA without treatment (control) and TT= total fungal growth on PDA with LAB-CFS treatment.

D. Observation of Quantitative Antifungal Activity

a. Preparation of *Fusarium* Culture

Fusarium sp. cultures were grown on acidified Potato Dextrose Agar (PDA, Oxoid, CM 0139) and incubated at 28°C for 5 days following the method mentioned by [18] with modification. Sterilized distilled water (10 to 20 ml) was poured onto the PDA plates. Then, the fungal surface was gently scraped to loosen the spores and the spores suspension was collected. The spores suspension (1 ml at 10^5 spores/ml) were inoculated into 20 ml of malt extract broth (Oxoid, CM0057) and incubated for 5 day at 28°C in orbital shaker (PROTECH MODEL 722). Then, fungal cultures were homogenized in a sterilized blender for few minutes and used for further treatments.

b. Micro Titre Plate Assay

In malt extract broth (MEB) using 96 wells micro titre plates was inoculated with 10^5/ml of seven days fungal spores at ratio of 1:1 v/v (LAB CFS: spore suspension) incubated at 28°C for 72 h. Fungal growth in microtiter plates was observed at $_{630nm}$ using Elisa reader (EL 309, Biotek Instruments, Winooski, Vt.). The percentage growth inhibition of fungi was calculated using the equation; GI= [(TC- TT)/TC] x100; where GI refers to growth inhibitions (%), TC (%) = total OD_{630nm} in control and TT= total OD_{630nm} in treatment with LAB-CFS.

c. Effect of pH, Heating and Enzyme Treatments on Antifungal Activity of LAB-CFS

The pH of lactic acid bacterial cells free supernatants (LAB-CFS) were adjusted to pH 2,3,4, 5, 6, 7, 8 and 9

using 0.1 N HCl or 0.1 N NaOH, respectively. In another set of experiments, all LAB-CFS were heated at 80°C, 90°C in water bath and 121°C in the autoclave for 30 min and immediately cooled in ice water. The heat treated CFS were then tested against fungi using microtiterplate assay [19] with modification. For enzyme treatments, pepsin (pH 1.5), and papain (pH 6.5) and proteinase K (pH 7.5) (Sigma) at a concentration of 0.1 mg enzyme/ml was used following the method of [20] with modification. A ratio of 1:1 (CFS: fungal spore suspension) was inoculated into the micro titre plates and incubated at 28°C for 72 h. The fungal growth was measured at absorbance (OD_{630nm}) using Elisa reader (Micro titre plate Auto reader EL 309, Biotek Instruments, Winooski, Vt.). The percentage growth inhibition of fungi was observed using the equation; GI= [(TC- TT)/TC] x100; where GI refers to growth inhibitions (%), TC (%) = total OD_{630nm} in control and TT= total OD_{630nm} in treatment with LAB-CFS.

E. Data Analysis

Mean ± standard deviation obtained from each analysis was analysed using One-way analysis of variance (ANOVA) and the means separation was done by the Tukey test at (P≤0.05). The statistical analyses were performed using Minitab 16 software.

III. RESULTS AND DISCUSSION

A. **Antifungal Activity of LAB Cells Free Supernatants Using Well Diffusion Method**

Based on the initial screening by dual overlay method all the LAB isolates were showed good antifungal activity against the *F. proliferatum*-LR was isolated from rose leaves as described in previous study [14]. Isolates *Lb. plantarum* 1FF and *Lb. plantarum* ATCC8014 showed better inhibitory activity and selected for further study with well diffusion method. In this method was used to evaluate the antifungal effect of the five selected cells free supernatants of lactic acid bacteria (LAB-CFS) on conidia germination and the mycelia growth of phytopathogenic fungi *F. proliferatum*-LR (Figure 1 & Table 1). It was observed that *F. proliferatum*-LR was inhibited by all lactic acid bacteria (LABs) with values ranged from 3.26 between 3.93 mm. However, the cells free supernatant (CFS) of *Lb. acidophilus* ATCC314 was showed higher clear zone compared to other CFS of LAB isolates after 48 h incubation. *In vitro* studies on LAB by [21-23] reported that some LAB isolates have antifungal activity against plant pathogens.

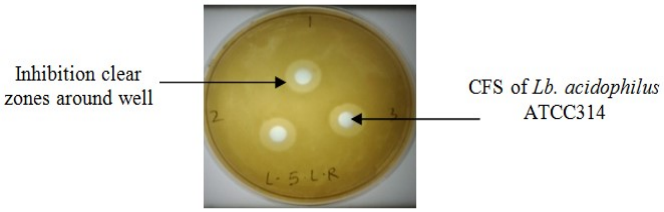

Figure 1: Antifungal activity of LAB-CFS against *F. proliferatum*-LR

Table 1. Antifungal activity of LAB strains against *F. proliferatum*-LR using well diffusion method

LAB isolates	Inhibitory clear zone (mm)
*Lb. plantarum*1MSS	3.76 ± 0.23^{ab}
*P. pentosaceus*1MSS	3.26 ± 0.23^{c}
Lb. acidophillus ATCC314	3.93 ± 0.05^{a}
Lb. plantarum ATCC8014	3.60 ± 0.10^{abc}
Lb. plantarum1FF	3.50 ± 0.10^{bc}

B**. Antifingal Activity Supernatant of Lactic Acid Bacteria on Fungal Growth Using Potato Dextrose Agar**

Lactic acid bacteria cells free supernatant (LAB-CFS) strains have been showed abibility to inhibit the fungal growth for example the antifungal activity of LAB-CFS to inhibit *F. proliferatum*-LR mycelial growth using potato dextrose agar (PDA) method is showed in Table 2.

Table 2. Inhibitory activity of LAB strains against *F. proliferatum*-LR using potato dextrose agar

LAB isolates	Inhibition on PDA (% mycelia growth in mm)
*Lb. plantarum*1MSS	32.9^{d}
*P. pentosaceus*1MSS	35.6^{c}
Lb. acidophilus ATCC314	36.1^{b}
Lb. plantarum ATCC8014	35.6^{c}
Lb. plantarum1FF	43.8^{a}

The results found that the inhibitory activity was significantly different (P≤0.05) by all CFS of LAB strains against *F. proliferatum*-LR after 5 days incubation. It was observed that

CFS of *Lb. plantarum1FF* strongly reduced the spreading of the *F. proliferatum*-LR about 43.8% compared to other CFS of LAB after 5 days incubation at 28°C in Figure 2.

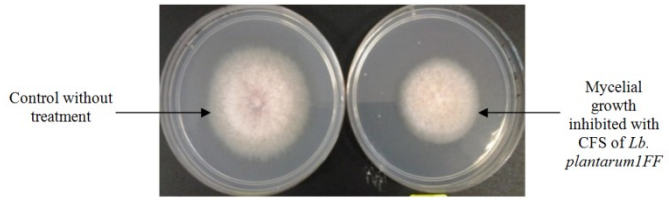

Figure 2: Mycelium growth inhibition of *F. proliferatum*-LR using *Lb. plantarum1FF*

Similarly, the antifungal activity of *Lb. plantarum* MYS6 was determined against a fumonisin producing identified fungus namely *Fusarium* proliferatum MYS9 when co-inoculation with the fungus on modified de Man Rogosa Sharpe medium revealed the inhibitory effect of *Lb. plantarum* MYS6 on fungal biomass and deformed hyphae growth [24]. Previously, it was confirmed *Fusarium* proliferatum is a carcinogenic agent because, it was showed ability to contaminate the food and known to colonize and produce fumonisin which is harmful chemical compound was suggested by the International Agency for Research on Cancer [25]. Interestingly, fumonisin emerged after discovering its high toxicity responsible for animal diseases like leukoence phalomalacia, porcine pulmonary edema as well as plant product such as common contaminant of maize and maize based products in worldwide [26].

C. Percentage Mass Reduction of Target Fungi by LAB CFS Using Micro Titre Plate Assay

Fungal bio-mass was greatly inhibited with longer incubation of fungi (72h) in MEB with added LAB-CFS (Table 3). The antifungal activity of *P. pentosaceus*1MSS supernantant significantly (P≤0.05) affected bio-mass growth.

Table 3. The percentage growth inhibition of *F. proliferatum*-LR by Cells free of lactic acid bacteria and incubated at 28°C for 72 h

LAB isolates	72h (%)
*Lb. plantarum*1MSS	93.79[a]
*P. pentosaceus*1MSS	94.65[c]
Lb. acidophilus ATCC314	94.56[bc]
Lb. plantarum ATCC8014	94.06[ab]
*Lb. plantarum*1FF	94.09[ab]

Thus, all method which were used for valuation of antifungal activity with CFS of LAB was showed strong destruction of *F. proliferatum*-LR which was reported to pathogenic fungi on chilli seeds suppression of germination and seedling systems [14].

D. Factors Influencing the Antifungal Activity of LAB-CFS

There were three different factors especially enzymes, heating and pH treatments showed influencing to antifungal activity of LAB-CFS against *F. proliferatum*-LR was isolated from leaves of rose ornamental plants as following.

E. Effect of Enzymes Treatments on LAB-CFS

Some proteolytic enzymes have ability to affect the antifungal activity of LAB supernatants; either decreased or increased the antifungal activity [27]. Crude LAB-CFS treated with pepsin, papain and protienase K significantly (P≤0.05) affected the percentages of spore germination densities as shown in Table 4. All the three proteolytic enzymes reduced the antifungal activity. Pepsin and papain increased the antifungal activity of all LAB-CFS except *Lb. plantarum* 1FF, while proteinase K decreased the antifungal activity of *P. pentosaceus* 1MSS. Similarly, it was reported by [28] supernatant of *Lb. plantarum* treated with proteinase K essentially reduced their antifungal activity. Additionally, [29] and [30] showed that the proteolytic treatment reduced the antifungal activity of *Lb. brevis* and *Lb. reuteri*R2.

Table 4. Percentage of mass growth inhibition against *F. proliferatum* by LAB supernatants treated with enzymes and incubated at 28°C for 72 h

LAB isolates	Pepsin	Papain	Protienase K
*Lb. plantarum*1MSS	70.10[a]	83.40[b]	83.60[bc]
*P. pentosaceus*1MSS	80.88[ab]	78.36[b]	58.88[a]
Lb. acidophilus ATCC314	63.85[a]	75.71[b]	74.97[b]
Lb. plantarum ATCC8014	86.70[ab]	82.11[b]	87.21[c]
*L. plantarum*1FF	54.15[a]	55.37[a]	84.11[bc]

F. Effect of Heating Treatments on LAB-CFS

Growth of *F. proliferatum* was reduced by 86.03 and 68.44 % in media containing CFS of *P. pentosacceus*1MSS and *Lb. plantarum* 1FF that was heat treated at 121°C for 30 min, respectively. However, other heat treated LBS-CFS maintained the antifungal activity with values > 90% similar to untreated CFS (Table 5). This indicates that the compounds with antifungal activity are heat stable. Similar observation was reported by [19] Muhialdin et al. (2011).

Table 5. Percentage of mass growth inhibition against *F. proliferatum*-LR by supernatants heated at 80°C, 90°C and 121°C and incubated at 28°C for 72 h

LAB isolates	Inhibitions		
	80 °C	90 °C	121 °C
Lb. plantarum 1MSS	93.79^{a}	92.56^{a}	90.23^{a}
P. pentosaceus 1MSS	94.65^{a}	93.37^{c}	86.03^{a}
Lb. acidophilus ATCC 314	94.56^{a}	93.18^{bc}	91.36^{a}
Lb. plantarum ATCC 8014	94.06^{a}	92.72^{ab}	91.16^{a}
Lb. plantarum 1FF	94.09^{a}	92.83^{abc}	68.44^{a}

G. Effect of pH Treatment on LAB Supernatants

Antifungal activity was noticed that to maintain when supernatants were adjusted at pH 2, 3, 4 and 5 Table 6. However, the fungal growth inhibition was diminished when the supernatants were adjusted to pH 6, 7, 8 and 9 as mentioned in Table 7. This indicates that the antifungal activity of LAB supernatant could be contributed by the undissociated organic acid [13]. Some cells free supernatants of lactic acid bacteria which were failed to inhibit the growth of fungi when changing the pH were indicated with g*= growth of *F. Proliferatum* showed no inhibition. Finally, it was obsereved that different factors such as heat, enzymes and pH treatment influencing the antifungal activity of LAB-CFS after 72 h incubation.

Table 6. Percentage of mass growth inhibition against *F. proliferatum*-LR by supernatants at pH 2 to 5 and incubated at 28°C for 72 h.

LAB isolates	pH-2	pH-3	pH-4	pH-5
Lb. plantarum 1MSS	91.32^{b}	85.76^{a}	89.14^{c}	51.58^{a}
P. pentosaceus 1MSS	87.01^{a}	84.24^{a}	76.23^{a}	60.88^{a}
Lb. acidophilus ATCC314	87.37^{ab}	88.92^{a}	87.24^{bc}	41.76^{a}
Lb. plantarum ATCC8014	86.69^{ab}	87.85^{a}	84.48^{b}	43.68^{a}
Lb. plantarum 1FF	86.89^{ab}	88.10^{a}	87.09^{bc}	82.90^{a}

Table 7. Percentage of mass growth inhibition against *F. proliferatum*-LR by supernatants at pH 6 to 9 and incubated at 28°C for 72 h.

LAB isolates	pH-6	pH-7	pH-8	pH-9
Lb. plantarum 1MSS	8.51^{b}	g^{*a}	g^{*a}	30.61^{a}
P. pentosaceus 1MSS	g^{*a}	11.26^{a}	g^{*a}	20.36^{a}
Lb. acidophilus ATCC314	6.82^{b}	g^{*a}	g^{*a}	15.50^{a}
Lb. plantarum ATCC8014	g^{*a}	g^{*a}	g^{*a}	18.65^{a}
Lb. plantarum 1FF	g^{*a}	g^{*a}	g^{*a}	16.75^{a}

IV. CONCLUSION

The LAB used in this study showed good antifungal activity using the method such as well diffusion, on PDA and micro titre plate against *F. proliferatum* that infected rose leaves. Heating and proteolytic enzyme treatments did not reduced the antifungal activity. The antifungal activity against *F. proliferatum* was effective at pH 2 to 5 and 9. This study also suggests that the LAB supernatant contains peptides that are stable to heat and acid.

V. ACKNOWLEDGEMENTS

Financial supports was provided by Department of Science and Technology, University Sains Islam Malaysia (USIM) and Ministry of Education Malaysia through Exploratory Research Grant Scheme (ERGS55087) are duly acknowledged.

VI. REFERENCES

[1]. Bai, G. and Shaner, G. 2004. Inn. Rev. Phytopath., 42: 135-16.

[2]. Alymanesh, M.R., Falahatirastegar, M., Jafarpour, B., Mahdikhanimoghadam, E. 2009. Genetic diversity in the fungus Fusarium solani f. sp. cucurbitae race 1, the casual agent of root and crown rot of cucurbits in Iran, using molecular markers. Pak. J. Biol. Sci., 12: 836-843.

[3]. Chehri, k., Salleh, B., Yli-Mattila, T., Reddy, K.R.N., Abbasi, S. 2011. Molecular characterization of pathogenic Fusarium species

in cucurbit plants from Kermanshah province, Iran. Saudi J. Biol. Sci., 18: 341-351.

[4]. Darnetty, D.M., Izzati, M.N.N.A., Azliza, M.Z.N., Izham, N.M and Salleh, I.N.M. 2008.

[5]. Elzbieta C., Lukasz S., Agnieszka W. 2015. Effect of environmental factors on Fusarium species and associated mycotoxins in maize grain grown in Poland, PLOS one doi:10.1371/journal. pone. 0133644.

[6]. Julian, A.M., Wareing, P.W., Phillips, S.I., Medlock, V.F.P. Macdonald, M.V. and Del Rio, L.E. 1995. Fungal contamination and selected mycotoxins in pre and postharvest maize in Honduras. Mycopath., 129: 5-16.

[7]. Chelkowski, J., Sinhaand, K.K. and Bhatnagar, D. 1998. Distribution of Fusarium species and their mycotoxins in cereal grains. In mycotoxins in Agr. and Food Safety, (Eds.). CRC Press, Marcel

[8]. Eckard, S. Wettstein, F.E., Forrer, H.R., Vogelgsang, S. 2011. Incidence of Fusarium species and mycotoxins in silage maize. Toxins, 3: 949-967. CrossRefPubMed].

[9]. Azliza, I.N., Hafizi, R., Nurhazrati, M. and Salleh, B. 2014. Production of major mycotoxins by Fusarium species isolated from wild grasses in Peninsular Malaysia, Sains Malaysiana 43(1): 89-94.

[10]. Hajnalka, B., Bela, D., Szilvia F., Arpad, C. and Andras, S. 2017. Determination of mycotoxin production of fusarium species in genetically modified maize varieties by quantitative flow immune cyto-metrytoxins, 9, 70;doi:10.3390 / toxins 9020070,www.mdpi.com / journal / toxins.

[11]. Chitarra, G.S., Breeuwer, P., Nout, M.J.R., Van Aelst, A.C., Rombouts, F.M. and Abee, J.T. 2003. An antifungal compound produced by Bacillus subtilis YM10-20 inhibits germination of Penicillium roqueforti conidio spores, J. of App. Micro., 94-159.

[12]. Ogunbanwo, S.T., Fadahunsi, I.F., Molokwu, A.J. 2014. Thermal stability of lactic acid bacteria metabolites and its application in preservation of tomato pastes, Malaysian J. Micr., 10(1):15-23.

[13]. Laref, N., Guessas, B. 2013. Antifungal activity of newly isolates of lactic acid bacteria, Inn. Roma. Food Biotech., 13.

[14]. Husain, A. Zaiton, H. Nur, H.-F., Nizam M. L. 2017. Antifungal activity of lactic acid bacteria isolated from soil rhizosphere on Fusarium species infected chilli seeds. American Sci. Res. J. .Eng., Tech., and Sci. (ASRJETS) Volume 29, No 1, pp 182-202

[15]. Muhialdin, B.J.A. and Hassan, Z. 2011. Screening of lactic acid bacteria for antifungal activity against Aspergillus oryzae", American J. App. Sci., 8(5): pp. 447-451, ISSN 1546-9239.

[16]. Magnusson J., Schnurer J. 2001. Lactobacillus coryniformis subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound, App. Env. Micr., 67:1-5.

[17]. Nene,Y. L., Thapliyal, B. N. 1973. Fungicides in plant diseases control, 3rd edition, Oxfordand IBH Publishing Co. Pvt. Ltd., New Delhi., 3: p.325.

[18]. Hamed, H., Yomna, A. M. and Shadia, M. A. A. 2011. In vivo efficacy of lactic acid bacteria in biological control against Fusarium oxysporum for protection of tomato plant, Life Sci. J. 8(4): pp. 462-468.

[19]. Muhialdin, B.J., Hassan, Z., Sadon, S., Zulkifli, N.A. and Azfar, A.A. 2011. Effect of pH and heat treatment on antifungal activity of Lactobacillus fermentumte007, Lactobacillus pentosusg004 and Pediococcus pentosaceuste 010, Inn. Romanian Food Biotech., 8:41-53.

[20]. Flora N.T., Kristina J.K., Svetla T., Danova, Svetoslav G., Dimov, and Farzad A.K. 2013. Probiotic properties of endemic strains of lactic acid bacteria, J. of BioSci. Biotech., 2(2):109-115.

[21]. El-Mabrok, Zaiton H., Ahmed M.M. And Khaled M.A.H. 2012. Screening of lactic acid bacteria as bio-control against (Collectotrichum capsici) on Chilli Bangi. Res. J. of App. Sci., 7, 9 (12): 446-473.

[22]. Rosalia, Tlluis, B. Emilio, M. Esther B. 2008. Lactic acid bacteria from fresh fruit and vegetables as bio-control agents of phyto-pathogenic bacteria and fungi, Inn. Micr., 11: 231-236.

[23]. Wang, H. Yan, Shin, H. 2011. Activity against plant pathogenic fungi of Lactobacillus plantarum IMAU10014 isolated from Xinjiang Koumiss, J. Ann. Micr., 61: 879-885.

[24]. Deepthi, B.V., Poornachandra, R. K., Chennapa, G., Naik M.K., Chandrashekara, K.T., Sreenivasa, M.Y. 2016. Antifungal attributes of Lactobacillus plantarum MYS6 against fumonisin producing Fusarium proliferatum associated with poultry feeds. plos one 11(6): e0155122. doi:10.1371/j.pone.0155122.

[25]. The International Agency for Research on Cancer (IARC). 2002. Monographs on the evaluation of carcinogenic risks to humans, some traditional herbal medicines, some mycotoxins, naphthalene and styrene., 82.
[26]. Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O., Wingfield, M.J. 2005. Natural occurrence of Fusarium and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. PLoS ONE., 99: 173-183.
[27]. Atta, H.M., Refaat, B.M. and El-Waseif, A. A. 2009. Application of biotechnology for production, purification and characterization of peptide antibiotic produced by probiotic Lactobacillus plantarum, NRRL B-227, Global J. of Biotech. & Biochem., 4(2): 115-125.
[28]. Rouse, S., Harnett, D., Vaughan, A., Sinderen, D.V. 2008. Lactic acid bacteria with potential to eliminate fungal spoilage in foods, J. App. Micr., 104: 915-923.
[29]. Mauch, A., Bello, F.D., Coffey, A. and Arendt, E.K. 2010. The use of Lactobacillus brevis PS1 to in vitro inhibit the outgrowth of Fusarium culmorumand other common Fusarium species found on barley. Int. J. Food Micr.,141: 116- 121.
[30]. Guo, J., Mauch, A., Galle, S., Murphy, P., Arendt, E.K., and Coffey, A. 2011. Inhibition of growth of Trichophytontonsurans by Lactobacillus reuteri, J. App. Micr., 111: 474-483.