In Vitro Efficacy Of Lactic Acid Bacteria With Antifungal Activity Against Fusarium Sp. CID124-CS Isolate From Chilli Seeds

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Abstract: Lactic acid bacteria (LAB) are known as to have inhibitory activity against fungi and other pathogens. In this study LAB from soil and fermented chilli fruits were evaluated for their inhibitory activity against Fusarium sp. CID124-CS that was isolated from chilli fruits. Three LAB isolates (Lb. plantarum1-MSS, P. pentoceous1-MSS isolated from soil, one Lb. plantarum1-FCF from fermented chilli) and two from ATCC culture Lb. acidophilus ATTCC314 and Lb. plantarum ATCC8014 showed strong inhibitory activity against growth of target fungi evaluated by well diffusion assay showed high growth inhibition (6.05 mm to 7.60 mm) within 48 h at 28^{o}C. Adding LAB supernatant to Potato Dextrose Broth (PDA) with fungi reduced mycelia growth from 36.00% to 60.00%. Similarly, fungal mass reduction with cells of LAB in De Man Rogosa and Sharpe Broth (MRSB) ranged 98.0% to 99.9% after 72 h incubation at 28^{o}C by micro tire plate assay. Whereas, conidial germination in MEB with LAB supernatants were reduced by 93.3% to 96.6% using micro titre plate assay. This study showed that cells and CFS of LAB isolated from soil have antifungal activity and could be used as antifungal agent against Fusarium sp. CID124-CS that infect to chilli.

Keywords: Antifungal Activity, Fusarium sp. CID124-CS, Lactic Acid Bacteria (LAB), Rhizospheric soil

1. INTRODUCTION

Microorganisms lactic acid bacteria (LAB) are easily isolated from fermented foods, but recently novel sources of LAB strains were isolated from the soil that can be used as control soil borne phytofungi [1]; [2]. LAB produce a variety of antimicrobial compounds and effective substances such as lactic, acetic, propionic acids, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide [4]. The use of LAB is not limited to the production of fermented foods but can be used as bio-control agents in plants [5]. In vitro studies indicate that LAB has potential application as bio-control against phyto-pathogenic fungi [6]; [7]; [8]; [9]; [10]. The approach of using microbes to control growth and proliferation of phytofungi is desirable since use of chemicals and fungicides in agriculture would create environmental pollution, and thus their use should be reduced or avoided. Additionally, certain microbes known as plant growth promoting bacteria (PGPB) such as LAB can function both as bio-control and plant growth regulator [5]. Fusarium solani is the causetive agent of Fusarium root rot diseases [11] in many vegetable crops worldwide and decrease the quantity and quality of major world crops and others economical plants [12]; [13]. Infection by Fusarium sp. are main cause of plant diseases in many countries for instances, in Italy [14], Saudi Arabia [15], Portugal [16] and Turkey [17]. Fusarium sp. also produce mycotoxin (trichothecene, zearalenone and fumonisin) which can be present in infected plants. Most the physical and chemical methods are applied for the detoxification of agricultural products contaminated with mycotoxins is restricted because of problems concerning safety issues, feasible losses in the nutritional quality of treated commodities, coupled with limited efficacy and cost implication [1]. Therefore, LAB with its reported antifungal activity [18] is considered one of the most prominent non-pathogenic strains that can be used s bio-control agent against Fusarium sp. CID124-CS. This work reports the in vitro study of cells and CFS of LAB isolated from soil and other fermented foods were used as biocontrol agent agaist phytopathogenic fungi especially Fusarium sp. CID124-CS that was isolated from chilli fruits.

2. MATERIALS AND METHODS

2.1 Preparation of Cells Free Supernatant from Lactic Acid Bacteria

The lactic acid bacteria (LAB) Lactobacillus plantarum 1MSS, Pediococcus pentosaceus 1MSS, Lb. plantarum1FF, Lb. acidophilus ATCC 314 and Lb. plantarum ATCC 8014 isolates were inoculated into de Man Rogosa and Sharpe Broth (MRSB Oxoid, CM0359) and incubated for 24 h at 37°C in aerobic shaker incubator (SASTEC Laboratory Equipment, Malaysia) using method described by [18]. The LAB-CFS was prepared by centrifuging the broth at 11500 rpm for 10 min at 4^{o}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).

2.2 Preparation of spore suspensions of phyto-pathogenic fungi

Fusarium sp. CID124-CS cultures was grown on acidified Potato Dextrose Agar (PDA, Oxoid, CM 0139) and incubated at 28°C for 5 days following the method mentioned by [5] 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^{o}C in orbital shaker (PROTECH MODEL 722). Then, fungal cultures were homogenized in a sterilized blender for few minutes and used for further treatments.

2.3 Inhibitory activity against Fusarium sp. CID124-CS 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 [20] with modification. In this method one ml spore suspension

(10^5/ml) from five days old Fusarium sp. CID124-CS 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.

2.4 Inhibitory activity against Fusarium sp. CID124-CS 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 of Fusarium sp. CID124-CS was carefully placed in the centre of the Petri dishes and incubated at room temperature for 5 days [21] 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.

2.5 Inhibitory activity lactic acid bacteria using Micro titre plate assay

Evaluation for antifungal activity by LAB were determined by mixing fungal spore suspension (100µL) and LAB in MRS 20mL of De Man Rogosa and Sharpe Broth (MRSB-OXOID CM0359 (MRSB) following the method described [5] with modification. The overnight cells of lactic acid bacteria 100µL was poured to 100 µl fungi Fusarium sp. CID124-CS suspension (1:1 v/v) in micro titre plate wells. Similarly**, s**even days old Fusarium sp. CID124-CS was grown in MEB, then 1mL was serially diluted. After that 100 µL of fungi suspension was poured to 100 µL supernatant (1:1 v/v) in micro titter plate and incubated at 28^0C for 72 h. Then growth of fungi was observed at OD_{630nm} by Elisa reader (BIOTEX). The percentage of inhibition was calculated using the equation; GI= [(TC-TT)/TC] ×100; where GI: growth inhibitions (%), TC= total fungal mass (control) and TT=total fungal mass with LAB.

2.6 Statistical analyses

All data were analyzed by one-way analysis of variance (ANOVA) and by the Tukey test, the statistical significance (p≤0.05) program from Minitab 16 software was used.

3. RESULTS

A total of 21 lactic acid bacteria (LAB) isolates were isolated and three ATCC cultures were screened for antifungal activity using dual overlay against fungi was isolated from chilli seeds (CS) showed typical colony morphology on PDA mycelia and characteristic spores of Fusarium sp. CID124 and detail description has been mentioned in previously publication (Akaram et al. 2017). Based on screenig five LAB strains was selected which showed variable antifungal activity against Fusarium sp. CID124-CS. isolates Lb. plantarum1-MSS, Pediococcus pentosus1; one from fermented chilli fruits Lb. plantarum1-FCF, Lb. acidophilus ATCC314 and Lb. plantarum ATCC8014.

3.1 Inhibitory activity of LAB cell free supernatants using well diffusion method

Well diffusion method was used to evaluate the inhibitory effect of the five selected LAB-CFS on conidia germination and the mycelial growth significantly (P≤0.05) of phytopathogenic Fusarium sp. CID124-CS was isolates from chilli seeds (Figure 1). It was observed that fungi Fusarium

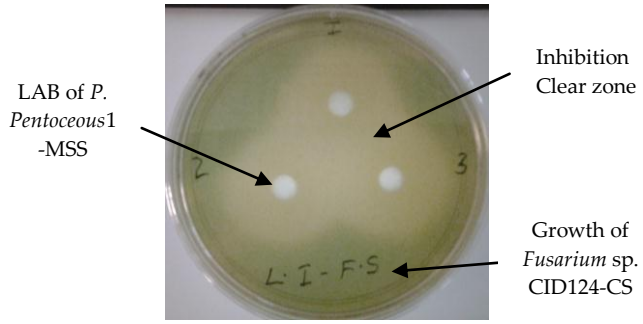

Fig-1: *Inhibitory activity of LAB-CFS against Fusarium sp. CID124-CS as evaluated by well diffusion method*

sp. CID124 was inhibited by all LABs with values ranged from 6.05 between 7.60 mm after 48 h incubation. However, mycelial growth of Fusarium sp. CID124-CS was inhibited by P. pentoceous-MSS, Lb. Plantarum1-MSS and Lb. Plantarum ATCC8014 against Fusarium sp. CID124-CS isolates in (Table1) ranges 6.06, 6.40 and 7.05 mm respectively.

Table-1: *Inhibitory activity of LAB-CFS evaluated against against Fusarium sp. CID124-CS using well diffusion*

LAB Isolates	inhibition zones (mm)
Lb. Plantarum1-MSS	6.40^{ab}
P. pentoceous1-MSS	6.06^{b}
Lb. acidophilus ATCC314	6.05^{ab}
Lb. plantarum ATCC8014	7.05^{a}
Lb. Plantarum1-FCF	7.60^{a}

3.2 Inhibitory activity supernatant of lactic acid bacteria on fungal growth using potato dextrose agar

The antifungal activity of LAB-CFS to inhibit Fusarium sp. CID124-CS mycelial growth using PDA method is showed in Table 2. The results found that the inhibitory activity was significantly different (P≤0.05) against all Fusarium sp. CID124-CS after seven days incubation. It was observed that LAB-CFS of P. pentoceous1-MSS isolated from soil strongly has been reduced the spreading of the Fusarium sp. CID124-CS about 60.0% after 7 d incubation at room temparature 28^oC. However, others LAB-CFS were showed good reduction and lowest reduction was noticed around

35.0 mm with CFS of Lb. Plantarum1-MSS against Fusarium sp. CID124-CS as described in Table 2.

Table-2: *Mycelium growth inhibition of Fusarium sp. CID124-CS using LAB-CFS evaluated on PDA after 7 d incubated at room temperature 28^{o}C*

LAB Isolates	Inhibition (%)
Lb. Plantarum1-MSS	35.0^{e}
P. pentoceous1-MSS	60.0^{a}
Lb. acidophilus ATCC314	40.0^{b}
Lb. plantarum ATCC8014	38.0^{c}
Lb. Plantarum1-FCF	36.0^{d}

3.3 Effect of cells and CFS of LAB against Fusarium sp. CID124-CS by micro titre assay

Antifungal activity of LAB cells was assayed with micro titre plate method in two different medium in MRSB and MEB. The effect of LAB cells and CFS did not inhibit significantly (P≥0.05) against growth of Fusarium sp. CID124-CS in Table 3. However, cells of LAB isolates were noticed to inhibit growth of spore germination of Fusarium sp. CID124-FCF higher than compared to LAB-CFS. The highest growth germination percentage of Fusarium sp. CID124-FCF was inhibited to 99.9% with cells of Lb. acidophilus ATCC314 and lowest germination percentage was recorded 97.0% with cells of Lb. plantarum ATCC8014 in MRSB medium. In contrast, to other LAB cells were showed slightly less inhibition against Fusarium sp. CID124-CS. Inhibition in MEB medium with all the LAB-CFS showed good inhibitory activity greater than 93.3% against the Fusarium sp. CID124-CS evaluated after 72 h incubation at 30^{o}C using micro titre assay. The CFS of Lb. plantarum1-MSS isolated from soil inhibited fungal spore germination of Fusarium sp. CID124-CS (96.6%). The CFS of P. pentoceous1-MSS and Lb. acidophilus ATCC314 showed good inhibitory activity against Fusarium sp. CID124-CS with percentage growth inhibition of 94.3% and 94.0%, in MEB medium respectively. In comparison to other LAB-CFS were showed slightly less inhibition against Fusarium sp. CID124-CS after 72 h incubation.

Table-3: *Percentage of growth inhibition of fungi Fusarium sp. CID124-CS by Cells and cells free supernatants (CFS) of lactic acid bacteria (LAB) after 72 h at 28^{o}C incubation*

LAB Isolates	Inhibition Activity (%)	
	Cells in MRSB	CFS in MEB
Lb. Plantarum1-MSS	99.0^{a}	96.6^{a}
P. pentoceous1-MSS	98.0^{a}	94.3^{a}
Lb. acidophilus ATCC314	99.9^{a}	94.0^{a}
Lb. plantarum ATCC8014	97.0^{a}	93.7^{a}
Lb. Plantarum1-FCF	99.5^{a}	93.3^{a}

4. DISCUSSION

Genotypically identified Fusarium sp. CID124-CS was isolated from chilli fruits has been described in previous study [19]. Fusarium spp. caused severe disease also known as Fusarium wilt disease in agriculture crops [22]; [23]; [24]; [25]. Becauase, fungal infection of F. solani was noticed that in chilli fruits and other plants [26] surprisingly, it was observed that certain strains of F. solani were reported to be resistant to common fungicide used [27]. Therefore, an alternative approach using LAB isolates as bio-control was attempted in many studies [28]; [29]; [30]. Similarly, 21 LAB isolated from different sources and three ATCC cutlurs especially Lb. acidophilus ATCC314, Lb. casei ATCC 334 and Lb. plantarum ATCC8014 were screen for antifungal activity as using dual overlay method mentioned in previous study was showed good antifunagla activity aginst Fusarium sp. CID124-CS [21]. In this study five selected LAB inhibited growth of funagal. Most report determined the antifungal activity of LAB from food sources. However, present study observed that LAB isolated from rhizophere soil also was abled to inhibit growth Fusarium sp. CID124-CS it was noticed that by using quantitative and qualititative methods. Differences in inhibitory activty was observed depending on the methods of evaluating the antifungal activity. Based on qualititative methods potato dextrose agar (PDA) method tends to indicate lower inhibitory activity than well method (Table 2). In addition, Lactobacillus sp., KUMBB001, KUMBB002, KUMBB003 & KUMBB005 were showed inhibitory activity against another pathogens especially, microorganisms Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumoniae MTCC 4030, Pseudomonas aeruginosa ATCC 27853 and Salmonella typhi MTCC 734 [31]. This indicates that the supernatant contains compounds that have antifungal activity as supported by results on mycelial mass growth inhibition experiments because LAB are known to produced many compound with antimicrobial activity such as lactic, acetic, propionic acids, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide [32]; [33]; [34]; [35]. However, the peptides, phenyllactic acids, are reported to be resposible for antifungal activity [36]. Based on quantitative study cells free supernatants of LAB isolates were showed strong inhibition in MRS broth compared to MEB broth using OD_{630nm} by Elisa reader (BIOTEX) in Table 3. Additionally, strains of Lactic acid bacteria were reported to inhibit spore germination of different pathogens especially F. oxysporum, Candida albicans and Bacillus subtilis [37]. Further work will concentrate on the characterization of the compound present in the cell free supernatant in LAB isolates were isolated from soil samples.

5. CONCLUSION

Lactic acid bacteria (LAB) isolates from soils samples were showed antifungal activity similar to as Lb. acidophilus ATTCC314 and Lb. plantarum ATCC8014 and fermented foods isolate Lb. Plantarum1-FCF. These LAB could be useful for the biocontrol against Fusarium sp. CID124-CS that infect chilli palants and other plants productivity. Further investigations to elucidate the nature of inhibiting compounds should be considered.

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7. REFERENCES

[1]. J. Kohl, J. Postma, P. Nicot, M. Ruocco and B. Blum, “Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria”, Biological Control, vol. 57, pp. 1–12, Dec. 2011.

[2]. P. L. Matthias, V. Michel., C. Martinez., C, Camps, “Lactic acid bacteria as bio-control agents of soil-borne pathogens”, Biological Control of Fungal and Bacterial Plant Pathogens IOBC-WPRS Bulletin, vol. 78, pp. 285-288, Jan. 2012.

[3]. A. Ouwehand, “Antimicrobial components from lactic acid bacteria,” In Lactic acid bacteria Microbiology and Functional Aspected Salminen, S Von Wright A. New York: Marcel Dekker Inc. pp.139-159, 1998.

[4]. H.A. Hamed, A. Yomna, Moustafa, Shadia and M. Abdel-Aziz, “In vivo Efficacy of Lactic Acid Bacteria in Biological Control against Fusarium oxysporum for Protection of Tomato Plant,” Life Science Journal, vol. 8, no. 4, pp. 462-468, Oct. 2011.

[5]. C. Stephane, D.J. Brion, N. Christophe, A. Cessaid, “Use of plant growth promoting bacteria for bio-control of plant diseases, principles, mechanism of action, and future prospects”, Appl. Envir. Microbiol., vol. 71, no. 9, pp. 4951-4959, Sept. 2005.

[6]. H. Ashgar and M. Pessarakli, “A review on biological control of fungal plant pathogens using microbial antagonists”, J. Biol. Sci, vol. 10, no. 4, pp. 273-290, 2010.

[7]. W. El-Mabrok, H. Zaiton, M.M. Ahmed and M.A.H. Khaled, “Screening of Lactic Acid Bacteria as Biocontrol Against (Collectotrichum capsici) on Chilli Bangi”, Research Journal of Applied Sciences7, vol. 9, no. 12, pp. 446- 473, 2012.

[8]. T. Rosalia, B. Lluis, M. Emilio and B. Esther, “Lactic acid bacteria from fresh fruit and vegetables as bio-control agents of phytopathogenic bacteria and fungi”, Int. Microbiol. Vol. 11, pp. 231-236, Oct. 2008.

[9]. H.Y. Wang, J.H. Shin, Activity against plant pathogenic fungi of Lactobacillus plantarumIMAU10014 isolated from Xinjiang Koumiss. Ann. Microbiol. vol. 61, pp. 879-885. 2011. Doi: 10.1007/ s 13213-011-0209.

[10]. A.M. Taleb, Amira, E. Kadriya and O. Fatimah, Al-Otibi, Assessment of antifungal activity of Rumex vesicarius L. And Ziziphus spina-christi (L.) wild extracts against two phytopathogenic fungi. African Journal of Microbiology Research, vol. 59, no. 9, pp. 1001-1011, May, 2011.

[11]. S. Parveen, S.E. Haque and A. Ghafar, “Biological control of Meloidogynejavanica on tomato and okra in soil infected with Fusariumoxysporium”, Pak. J. Nematology, vol. 11, pp. 151-156, 1993.

[12]. A. Ghafar, “Biological control of root rot and root knot disease complex ofvegetables”, Final Research Report, Dept. Bot. Univ, Karachi, pp: 725-770, 1995.

[13]. G. Polizzi, D. Aiello, V. Guarnaccia, A. Vitale, G. Perrone and G. Stea, “First report of Fusarium wilt of Paper Flower (Bougainvillea glabra) caused by Fusarium oxysporum in Italy”, Plant Disease, vol. 94, no. 4, pp. 483.1-483.1, 2010.

[14]. K. Perveen and N. Bokhari, “First report of Fusarium wilt of Lavandula pubescens caused by Fusarium oxysporum in Saudi Arabia”, Plant Disease, vol. 94, no. 9, pp. 1,163.2 - 1,163.2, Sept. 2010.

[15]. M. Felgueiras, A. Dias, G. Chicau, M. Berbega, M. Leon, and J. Armengo, “First Report of Fusarium wilt caused by fusarium oxysporum f. sp. basilici on Ocimum minimum in Portugal”, Plant Disease, vol. 94, no. 9, pp. 1,170.1 - 1,170.1, 2010.

[16]. G. Karaca, and E. Kahveci, “First report of Fusarium oxysporum f. sp. radicis-cucumerinum on cucumbers in Turkey. Plant Pathology, vol. 59 no. 6, pp. 1173–1174. 2010.

[17]. B.J.M. Muhialdin and H. Zaiton, “Antifungal Activity of Lactobacillus fermentum Te007, Pediococcus pentosaceus Te010, Lactobacillus pentosus G004, and L. paracasi D5 on selected foods”, Journal of Food Science, vol. 76, no. 7, pp. 493-499, Sep. 2011.

[18]. A. Husain, H. Zaiton, H.-F. Nur and M.L. Nizam, “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**)** vol. 29, no 1, pp. 182-202, Apr. 2017.

[19]. J. Magnusson, and J. Schnurer, “Lactobacillus coryniformis subsp. coryniformis strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Applied Environ. Microbiol. vol. 67, no. 1 pp. 1-5. DOI: 10.1128/AEM.67.1.1-5. Jan. 2001.

[20]. Y.L. Nene, and B.N. Thapliyal, “Fungicides in plant diseases control, 3rd edition”, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi,p.325, Jan. 1973.

[21]. H. Suga, Y. Hirayama, M. Morishima, T. Suzuki, K. Kageyama and M. Hyakumachi, "Development of PCR Primers to Identify Fusarium oxysporumf. sp. Fragariae", Plant Disease vol. 97, no. 5, pp. 619-625, May, 2013.

[22]. X. Fang, P.M. Finnegan, M.J. Barbetti, "Wide Variation in Virulence and Genetic Diversity of Binucleate Rhizoctonia Isolates Associated with Root Rot of Strawberry in Western Australia", PLoS ONE, vol. 8 no. 2, e55877. doi:10.1371/ journal. pone. 0055877, Feb. 2013.

[23]. S.J. Kamil, H.A. Hurria and B.A. Saba, "Fusarium wilt disease of strawberry caused by Fusarium oxysporum f. sp. fragariae in Iraq and its control", Journal of Experimental Biology and Agricultural Sciences, vol. 2, no.4, Aug. 2014.

[24]. Y. Ebihara and S. Uematsu, "Survival of strawberry-pathogenic fungi Fusarium oxysporum f. sp. Fragariae, Phytophthora cactorum and Verticillium dahliae under anaerobic conditions", Journal of General Plant Pathology vol. 80, pp. 50-58, 2014.

[25]. S. Zivkovic, S. Stojanovic, Z. Ivanovic, V. Gavrilovic, T. Popovic, and J. Balaz, "Screening of antagonistic activity of microorganisms against Colletotrichum acutatum and Colletotrichum gloeosporioides", Archives of Biological Science Belgrade, vol. 62, pp. 611- 623, 2010.

[26]. H.H.E. Karima, "Effect of some fungicides on Fusarium solani and Rhizoctonia solani in bean plants", Egyptian Journal of Applied Sciences, vol. 23 no. 10B pp. 668 – 687, 2008.

[27]. M. Kivanc, S.A. Kivanc and S. Pektas, "Screening of Lactic Acid Bacteria for Antifungal Activity against Fungi", J. Food Process Technol. Vol. 5, no. 3, p. 310. Apr. 2014. doi:10.4172/2157-7110.1000310.

[28]. E. Sevgi and I. Tsveteslava, "Antifungal activity of lactic acid bacteria, isolated from Bulgarian wheat and rye flour Journal of Life Sciences", vol. 9 pp. 1-6, Jan. 2015. doi: 10.17265/1934-7391/2015.01.001

[29]. N.T. Leopold, T. Roger and M.M.F. Carl," Antifungal activity of lactic acid bacteria against molds isolated from corn and fermented corn paste," American Journal of Microbiological Research, vol. 4, no. 4, pp. 90-100, 2016.

[30]. V.R. Usha and B.V. Pradeep, "Isolation, identification and characterization of a novel probiotic strain (lactobacillus paracasei kumbb005) from cow milk samples and its antibacterial activity," World Journal of Pharmaceutical Research, vol. 3, NO. 5, pp. 599-612, Jun. 2014.

[31]. S. Jagoda, K. Blazenka, B. Jasna, L.P. Andreja, H. Ksenija and M. Sresko, "Antimicrobial Activity of Lactic Acid Bacteria", Food Technol. Biotechnol. vol. 48, no. 3, pp. 296–307, May. 2010.

[32]. A. Chowdhury, N. Hossain, N.J. Mostazir, Fakruddin, M. Billah and M.M. Ahmed, "Screening of Lactobacillus spp. from buffalo yoghurt for probiotic and antibacterial activity", J. Bacteriol and Parasitol, vol. 8. pp. 156, Oct. 2012.

[33]. T. Islam, F. Sabrin, E. Islam, M. Billah, K.M.D. Islam, "Analysis of antimicrobial activity of Lactobacillus paracasei ssp. paracasei-1 isolated from regional yogurt", Int. Res. J. Applied Life Sci, vol. 1, no. 4, pp. 66-72, 2012.

[34]. H. Pyar and K. Peh, "Characterization and identification of Lactobacillus acidophilus using biology rapid identification system", Int. J. Pharm Pharm Sci, vol. 6 no. 1, pp. 189-193, Oct. 2014.

[35]. A.A. Sobowale, O.A. Odeyingb, H.O. Egberongbe, R.T. Feyisola, O.A. Ayinde, and A. Adesemowo, "Growth inhibition (in vitro) of Colletotrichum gloeosporioides isolated from cassava (Manihot esculenta) using Trichoderma longibrachiatum", African Journal of Microbiology Research, vol. 4, no. 21, pp. 2196-2201, Nov. 2010.

[36]. S.D. Kavita, D.S. Pragati, S.J. Ashvini and C.A. Pavan, "Lactic Acid Bacteria: Antimicrobial activity and in vitro, in vivo studies of LAB activity on Fusarium oxysporum infected tomato seeds", Int. J. Advanced Res., vol. 3, no. 5, pp. 954-963, May. 2015.