Study the Effect of *Lactobacillus spp*. on the growth of *Trichophyton rubrum*

Hana K. Maikhan[a] , Khalid A. Habeb[b], Shwan K. Rachid[C]

[a]B.Sc., M.Sc., Ph.D. student, Department of Biology, College of Education,University of Garmian\ Kurdistan region of Iraq.

[b]B.Sc., M.Sc., Ph.D. Professor in microbiology, Department of Biology,College of Science for women\ University of Baghdad, Iraq.

[C]B.Sc., M.Sc., Ph.D. Professor in molecular biology, Assistant Chief of Charmo University, Kurdistan region of Iraq .

Abstract

Background: *Trichophyton rubrum* is one of dermatophytes that responsible for the majority of dermatophytosis worldwide. *Lactobacillus* is the largest genus of lactic acid bacteria (LAB) which can be used as probiotic and produced antifungal compounds. The present study aimed to evaluate the effect of some *Lactobacillus spp.* on the growth of fungal pathogen *T. rubrum*.

Materials and Methods: Atotal of 50 samples of infected hair, nail and skin were isolated from patients with dermatophytosis, five clinical isolates were diagnosed as *Trichophyton rubrum* based on conventional laboratory and molecular approach identification which including gene-specific PCR for amplified ITS gene resulted in 690bp and PCR-RFLP which employs *BstN1* restriction enzyme resulted in distinguish bands . A total of 25 different samples, were obtained three species of *Lactobacillus* including *L. plantarum; L. pentosus* and *L. brevis* which identified according to the colonial morphology on MRS agar, microscopic examination and biochemical test by API 50 CHL kit. Antagonistic activities of both CFS and entire cells of *Lactobacillus* species on *T. rubrum* by agar well diffusion and MIC revealed highest inhibition activity by *L. plantarum* followed by *L. brevis* and *L. pentosus* respectively. The antagonistic ability of entire cells was highest than CFS. Phenylacetic Acid (PLA) as antifungal compound was determined by HPLC-UV from Lactobacilli species extraction and showed higher concentration in *L. plantarum* about 19.6 ppm followed by *L. brevis* and *L. pentosus* (17.2 and 14.1 ppm respectively) in comparing with the standard 3 ppm.

Conclusion: Our study investigated the activity of the antifungal compounds in the CFS of *Lactobacillus* species with lowest activity than the entire cells and the strongest activity was caused by *L. brevis* followed by *L. plantarum* and *L. pentosus* respectively. The maximal antifungal compound PLA was found in the CFS of *L. plantarum.*

KEYWORDS: *Trichophyton rubrum, Lactobacillus plantarum, Lactobacillus brevis, L.pentosus,* antagonistic activity.

I. Introduction

Trichophyton rubrum is the most prevalent type of dermatophyte which infects keratinized tissue in human such as hair, skin and nail (Cervelatti *et al.*, 2004). In

addition this species has the ability to avoid cell mediated immunity and therefore 80% of the nail infection (onychomycosis) and 90% of the chronic infection of dermatophyte caused by *T. rubrum* (Baeza *et al.*, 2006; Baeza *et al.*, 2007). Identification and characterization of *T. rubrum* can be achieved by two methods in general, conventional method based on the macroscopic and microscopic examination. In addition the conventional method still based on the results of microscopic examination particularly (macroconidia, microconidia and hyphal properties) and colony properties when growing on SDA (Kane *et al.*, 1997). The second method for identification based on molecular approaches which have been developed to provide more sensitive and rapid method to the identification of dermatophytes which include gene specific PCR, RFLP PCR, and sequencing of Internal Transcript Spacer ITS region (Gräzer *et al*., 1999).

Lactobacillus is an important genus of LAB with its fermentative activity and nutritional benefit (Gilliand, 1990). Furthermore, *Lactobacillus* species have the ability to ferment 85-90% of the sugar and produce lactic acid which inhibits the growth of the pathogenic microbes and favors the growth of Lactobacilli which can grow at low pH (Siegrist, 2010). The most important species of *Lactobacillus* which can be used as probiotics *L. reuteri, L. casei, L. acidophilus, L. brevis, L. pentosus, L. plantarum, L. fermentum, L. rhamnosus, L. paracasei, L. gasseri* and *L. mucosa (* Gue *et al.*, 2011). According to the fermentation ability of hexose, *Lactobacillus* can be classified into three main groups: obligate homofermentative which ferment hexose and form lactic acid, facultative homofermentative which ferment hexose which only form lactic acid or together with acetic acid and ethanol and obligate heterofermentative Lactobacilli which ferment hexose and form lactic acid, acetic acid, CO_2 and ethanol, on the other hand, the fermentation of pentose produce lactate and acetate (Goyal *et al*., 2012). Number of antibacterial compounds particularly bacteriocin produced by LAB (Piper *et al.*, 2009). While the studies on antifungal compounds that produced by LAB is limited. A number of low molecular weight compounds mostly organic acid have the ability to eliminate the fungal growth (Ryan *et al.* 2008).

II. Materials and Methods

Isolation of *Trichophyton rubrum*: A total of 50 suspected patients with dermatophytosis were identified by the supervision of specialized dermatologist, in the derma unit of the General hospital in Kalar district/ Sulaimania province\Iraq. After direct microscopic examination by KOH, the remaining samples cultured on SDA supplemented with cyclohexamide (500 mg\L) and chloramphenicol (50 mg\L) and incubated at 25-30 °C for 1-2 weeks. The developing colonies were examined morphologically: colony surface and reveres, microscopically: macro and microconidia and biochemically, hair perforation test growth on rice grain and urease test (Soon and Adam, 1978; Evans and Retchardson, 1989). Molecular identification of *T. rubrum* was made by extraction of the fungal DNA using fungal extraction DNA kit from (OMEGA\USA) according to the leaflet processed by the company, then the gene-specific PCR technique used to amplify the ITS region of 5.8S gene in rDNA by using set primers ITS1 and ITS4 (White, 1990). 25 µL of PCR reaction mixture was prepared containing: 12.5 µL of PCR master mix (GeNet Bio\Korea); 2 µL of DNA template; 0.5 µL of each primer and the remaining volume completed with deionized water (Li *et al.*, 2008). The PCR conditions consist of: initial denaturation 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 seconds; annealing at 55 °C for 30

seconds; extension at 72 °C for 1 min and final extension at 72 °C for 4 min (Refai*et al.*, 2013).

Identification of *T. rubrum* was performed by using RFLP PCR analysis which carried out by *BstN1* (BioLab\UK) restriction enzyme. The PCR products were obtained previously subjected to digestion by incubating 10 µL of PCR product, 0.5 µL of *BstN1* enzyme, and 1.5 µL of 10 X NEB buffer to a final volume 12 µL and incubated at 60 °C in in dry oven for an hour according to the leaflet processed by the company. All amplicons PCR and digested products were electrophoresed in a 1.5 and 2 % agarose gel in TBE buffer at 90 V for 60 min. The DNA bands were obtained by gene specific PCR and the fragments were digested by *BstN1* enzyme compared with profiles obtained by Jackson *et al.*, (1999).

Isolation of *Lactobacillus spp.*: 25 samples collected from different source included healthy infant stool (1 month-6 month), vaginal swabs from healthy women, breast milk, cow milk, cow yogurts, sheep milk, sheep yogurts, deer milk and goat milk. All isolates (with the exception of vaginal swabs which directly cultured on MRS agar) were cultured on 50 ml of MRS broth and incubated at 37 °C for 24 hour in microaerophilic condition. Each sample was sub cultured on two plates of MRS agar, one incubated aerobically and the other was incubated anaerobically at 37 °C for 24-48 hour (Pelinescu *et al.*, 2009). Identification of the genus *Lactobacillus* was carried out on the basis of morphological characters, Gram's stain, microscopic examination and biochemical testes: catalase and oxidase testes (Hoque *et al.*, 2010). API 50 CHL kit(bioMérieux®, France) used to identify the species of *Lactobacillus* dependent on the fermentation of 49 carbon source present in the kit as strips according to the leaflet of the kit. Furthermore, a probiotic capsule of *L. acidophilus* and *L. plantarum* (Pharma, UK) was used as a positive control.

Preparation of Bacterial Cell Free Supernatant (CFS): Colonies of *Lactobacillus* were grown on MRS agar inoculated into 250 ml of MRS broth with 5 ml of glycerol and incubated at 37 °C for 24 hours to prepare bacterial suspension with final concentration 1×10^8 CFU. Later bacterial cells were removed by centrifugation at 7200 g for 10 minutes. The obtained CFS was filtered through 0.45 µm Millipore filter (Cortés-Zavaleta*et al*., 2014).

Antagonistic activity: The antagonistic activities of all *Lactobacillus spp.* against *T. rubrum* were carried out by overnight culture both CFS and entire cells which applied by using agar well diffusion assay on Muller Hinton Agar (MHA) and MRS agar plates as follows:

A) CFS method: 100 µL of the diluted fungal suspension (10^6 CFU) was transferred into the surface of the (MHA) plate and spread with sterile swab then left for 30 minutes as triplicate. Three wells with 6 mm in diameter were punched on the surface of MHA plate by pasture pipette and filled with 60 µL of the CFS from each *Lactobacillus spp*. broth. All plates were incubated at 25 °C for 18-24 hours (Schillinger and Lucke, 1989). The positive result was observed as inhibition zone around the well. The experiment was repeated three times and the mean of inhibition zone was calculated with ± standard deviation and compared with the control (CFS of MRS broth).

B) Entire cells method: 100 µL of the diluted fungal suspension (10^6 CFU) was spread on the surface of MRS agar plate as previously. The wells filled with 60 µL

from overnight MRS broth containing 10^8 CFU of each *Lactobacillus spp.* Plates were incubated 37 °Cfor 24-48 hours under microaerophilic condition followed by additional incubation for 24-48 hours at 25 °C under aerobic condition to promote fungal growth (Guo, *et al.* 2011).

Determination of MIC and MFC:

The MIC and MFC of *Lactobacillus spp.* against the most prevalent type of dermatophytes were performed by microdilution assay as Kirby-bauer test (Clinical and Laboratory Standards Institute, 2012). The CFS of each species of *Lactobacillus* broth diluted with MHB to prepare serial dilution of antifungal agents with concentration 1000 µL\ml; 500 µL\ml; 250 µL\ml; 125 µL\ml; 62.5 µ\ml; 31.3 µL\ml and 15.6 µL\ml. A serial of sterile test tubes (nine tubes) prepared and numbered. 100 µL of the fungal suspension was mixed with 900 µL of Muller Hinton Broth (MHB) in test tubes from 1 to 9 , each tube was contained 10^6 CFU of fungal concentration. 1ml of each diluted CFS added into tubes 2-8. The first tube which used as positive control (only was contained 1ml of fungal pathogen 10^6 CFU and MHB) and the last tube which used as negative control was contained only 1ml of diluted CFS 1000µ\ml. All tubes incubated at 25 °C for 18-24 hours.

Identification of Phenylacetic Acid PLA in CFS:

For HPLC analysis, the cell free extract of each bacterium was a freeze dried samples then hydrolyzed in 6 N HCL at 100 °C. 1gm of CFE of each bacterium was dissolved in 1.5 ml of methanol then mixed with 1.5 ml of 6 N HCL and incubated at 100 °C for 4 hours. After cooling, the sample was extracted on the C_8 reversed phase disposable cartridges to remove the excess of HCL and the cartridges was activated by 2×2.5 ml of methanol then equilibrated with 2×2.5 ml of water. 1ml of hydrolyzed sample was applied and washed with 2.5ml of water then eluted with 2.5 ml of 0.05 M NaOH and collected. Then the solution subjected to the anion exchange extraction cartridge which activated with 2×2.5ml of methanol and equilibrated with 2×2.5ml of water. 2ml of the eluted fraction collected from the previous extraction were used and washed with 2×2.5ml of water and eluted with 2.5ml of 0.1 M phosphoric acid (H_3PO_4) and collected. 50 µL of eluted solution injected into the HPLC system which consist of C18-ODS (25 cm x 4.6 mm x 5 um) with detector UV-215 nm and flow rate 1.0 ml / min for detection of Phenylacetic acid (PLA). The concentration of the PLA in each sample was calculated according to the following formula:

$$C_{sam} = (C_{st} \times A_{sam} \backslash A_{st}) \times D.F \backslash wt$$

The mobile phase consist C_2H_3N and Orthophosphoric acid 25:75 ml. The calibration curve was obtained by the heights peak of PLA of the standard solutions which reported in a graph and the natural PLA content of the examined samples (Grossi *et al.*, 1988).

III. Results and Discussions

A total of 50 samples of infected hair, nail and skin isolated from patient were examined and cultured on SDA, five clinical isolates diagnosed as *T. rubrum* based on macroscopic morphology which characterized by white colonies at the surface and became creamy, suede like to downy, with red brown color at revers (Figure 1). Microscopically, there are numerous oval shape microconidia and cylindrical

macroconidia, as shown in Figure (2). Molecular identification of *T. rubrum* was made by gene- specific PCR to amplify ITS region of 5.8S gene in rDNA using pan fungal primers ITS1 and ITS4 and resulted band with 690bp. The results were obtained from PCR-RFLP of ITS region by *BstN1* restriction enzyme showed three distinct patterns bands 380, 180 and 100 bp (Figure 3). The profiles of electrophoretic analysis of patterns were obtained from ITS- RFLP in the clinical isolates showed similarity with those of Jackson *et al.,* (1999); Mochizuki *et al*., (2003); Elavarashi *et al.,* (2013); Al-Khafajii (2014) ; Ahmadi *et al.,* (2015); Ghojoghi *et al.,* (2015) who have identified dermatophytes by PCR RFLP and showed the same patterns of ITS RFLP. However, an important fact which observed in the present study during the performance of PCR- RFLP and differ from the previous studies, that the patterns bands were below 100 bp not revealed on the gel during the electrophoretic analysis and this is may be due to the small molecular weight of these packages bands and cannot be shown and needs to increase the molecular weight of the agarose gel. Consequently, the analysis of ITS region by *BstN1* endonuclease enzyme was provided simple method for dermatophytes characterization and this analysis showed the polymorphism of ITS region for strains and species of dermatophytes (Jackson *et al.*, 1999).

Based on the morphological characters on solid MRS agar medium, microscopic examination and biochemical testes, three isolates were obtained from different source identified as Lactobacillus. Further, a probiotic capsule comprised of two species *L. acidophilus* and *L. plantarum* used as a positive control. All isolates of *Lactobacillus* were gram positive, none spore forming and produced small size, spherical, white to creamy in color colonies on the surface of MRS agar medium. Microscopic examination showed rod to cocci shaped bacteria. Biochemically, catalase test is one of the most beneficial test for Lactobacillus identification which due to their simplicity. During performance of catalase test, no bubble formation indicated catalase negative and this enzyme doesn't mediated the decomposition of hydrogen peroxide H_2O_2 to produce O_2 (Pyar and Peh, 2013). Therefore, the bacteria utilize peroxidase to detoxify H_2O_2 and this enzyme doesn't release O_2 (Goyal*et al.,* 2012). In oxidase test, when a single colony of Lactobacillus was transferred to TMPD, no color changed into blue purple indicated the negative result because it doesn't produce cytochrome oxidase (Chowdhury *et al.,* 2012). According to Zourari *et al*., (1992) Lactobacilli are facultative anaerobic, couldn't synthesis porphyrins and consequently, they couldn't synthesis catalase and cytochrome oxidase.
The isolates were assigned as *Lactobacillus* further characterized by using API 50 CHL kit as *L. pentosus*, *L. plantarum* and *L. brevis* (Table 1)

Antagonistic activity:

In the present work, the antagonistic activity of *Lactobacillus* species against *T. rubrum* was studied by using bacterial cell free supernatant (CFS) and entire cells which carried by agar well diffusion assay on the surface of MHA and MRS agar medium. The results were obtained from incubation the bacterial CFS and *T. rubrum* at 25°C revealed that *L. plantarum* was the most efficient species which revealed inhibition zone reached to 28.00 ±1.00 followed by *L. brevis* and *L. pentosus*, were showed inhibition zones reached to 26.66 ± 0.57 and 26.00 ± 2.00 respectively (Figure 4) in comparison with the positive control which revealed inhibition zone reached to 17.33 ± 1.52. The study of antagonistic effect of entire cells from *Lactobacillus* species against *T. rubrum* appeared to be most efficient than the CFS.

The highest inhibition zone was recorded by *L. brevis* (83.66±0.57) followed by *L. plantarum* and *L. pentosus* were 81.66 ± 0.57, and 80.33 ± 0.57 respectively (Figure 5) comparing with the inhibition zone that recorded by the positive control (82.33 ± 1.52). The results were obtained from the study of antagonistic effect by using entire cells of *Lactobacillus* species against *T. rubrum* showed similarity with the results of Guo *et al*., (2011) and Guo *et al.,* (2012), who studied the antifungal effect of Lactobacilli entire cells on dermatophyte species such as *Microsporum canis, Epidermophyton flocosum* and *Trichophyton tonsurans* and reported that the entire cells of Lactobacilli species were more efficient than the CFS and revealed higher antifungal activity against dermatophytes.

Involvement of entire cells of *Lactobacillus spp.* as probiotics revealed better results than CFS this indicated that the antifungal compounds were produced by *Lactobacillus* species with a higher level in the presence of dermatophyte species (as direct contact between the probiotic and the fungal pathogen in the plate), perhaps this is due to that the direct contact, may stimulates the bacteria to produce larger amount of enzymes that inhibit the fungal growth. Gue *et al.,* (2012) found that *Lactobacillus reuterii* strains exhibited strong antifungal activity against human dermatophytes such as *Microsporum canis, M. gypseum , and Epidermophyton flocosum.* Another study showed that 20% of lactic acid bacteria strains exhibited antifungal activity against *T. tonsurans* (Gue *et al*., 2011). Several authors reported the detection of low molecular weight material with antifungal activity such as reuterin and phenylacetic acid which produced by *L .reuterii* (Nakanishi, 2002), *L. brevis* (Schütz and Radler, 1984), and *L. collinoidis* (Claisse and Lonvaud-Funel, 2000), and our results in agreement with these concepts in which *L. brevis* exhibited the strongest antifungal activity against fungal pathogen.

MIC and MFC:

The results were obtained from the study of MIC and MFC by Kirby-bauer test illustrated in table (2) and indicated that all three species of *Lactobacillus* showed antifungal activity against *T. rubrum*.

Based on the results were shown in table (2), the highest antifungal activity since their MIC and MFC values from the fermented CFS was revealed by *L. plantarum; L. brevis* and the positive control exhibited MIC value reached to 125 µL\ ml while the MFC value reached to 250 µL\ ml (Fig. 6) and this is correlate with the results were obtained from the study of antagonistic activity by using fermented CFS from *Lactobacillus* species by agar well diffusion assay and showed that *L. plantarum* was the most efficient species which revealed the highest inhibition zone on the surface of MHA medium against *T. rubrum*.

Detection of PLA by HPLC:

Phenylacetic acid partially purified from the CFS of each *Lactobacillus* species by HPLC-UV analysis. The chromatograms were obtained from standard solution and tested samples are shown in Figure (7). Retention time of PLA in the standard was 3.2-3.4 min. Then the PLA in the samples were detected at the same retention time. Concentration of the PLA in the samples compared with the concentration of the PLA in the standard which exhibit 3ppm. The concentration of the PLA in the samples is the highest than standard. The highest value of the PLA was 19.6 ppm in *L. plantarum* followed by *L. brevis* and *L. pentosus* (17.2 and 14.1 ppm respectively). PLA is an

antifungal compound produced as secondary metabolites. Lavermicocca *et al*. (2000) recorded the production of PLA from *L. plantarum*. Our results from Lactobacilli species (*L. brevis; L. pentosus* and *L. plantarum*) showed that these species containing high concentration of PLA and this is in agreement with the results of Magnossun, (2003) who reported that PLA is an active compound against yeast and molds when exist in high concentration, furthermore it act in synergy effect with other compounds which produced by *Lactobacillus spp*.as an overall antifungal compound.

References

- Ahmadi, B.; Mirhendi, H.; Shidfar, M.R.; Nouripour-Sisakht, S. Jalalizand, N.; Geramishoar, M. and Shokoohi, G.R. (2015).A comparative study on morphological versus molecular identification of dermatophyte isolates.J Mycol Med, 25(1):29-35.
- Al-Khafajii, K. (2014). Myco-epidemiologic and genetic study of rermatophytosis and non dermatophytes in middle euphrates Iraq. African Journal of Microbiology Research, 8(24): 2381-2386.
- Baeza, L.C.; Bailao, A.M.; Borges, C.L.; Pereira, M.; Soares, C.M.; Mendes-Gianni, M.J. (2007). cDNA representational difference analysis used in the identification of genes expressed by *Trichophytonrubrum* during contact with keratin. *Microbes. Infect*. **9**:1415-1421.
- Baeza, L.C.; Matsumoto, M.T.; Almeida, A.M.F. and Mendes-Giannini M.J.S. (2006).Strain differentiation of *Trichophytonrubrum* by randomly amplified polymorphic DNA and analysis of rDNAnontranscribed spacer. *J. Med. Microbiol*. **55:**429-436.
- Cervelatti, E.P.; Ferreira-Nozawa, M.S.; Aquino-Ferreira, R.; Fachin, A.L. and Martinez-Rossi, N.M. (2004).Electrophoretic molecular karyotype of the dermatophyte *Trichophytonrubrum. J.Genetics and Molecular Biology*.**27**(1):99-102.
- Chowdhury, A.; Hossain, M.N.; Mostazir, N.J.; Fakruddin, M. M. and Billah, M. (2012).Screening of *Lactobacillus spp.* from Buffalo Yoghurt for Probiotic and Antibacterial Activity.*J BacteriolParasitol***3**:156.
- Claisse, O. and Lonvaud-Funel, A. (2000). Assimilation of glycerol by a strain of *Lactobacillus collinoides* isolated from cider. *Food Microbiology***17:**513-519.
- Clinical and Laboratory Standards Institute Administrative Procedures, 2012).
- Cortés-Zavaleta,O; López-Malo, A.; Hernández-Mendoza,A and García, H.S. (2014). Antifungal activity of lactobacilli and its relationship with 3-phenyllactic acid production. *International Journal of Food Microbiology*. **173** .30–35.
- Eliane, P.; Cervelatti; Monica, S.; FerreiraN.;Roseli, A.; Ana, L.F.andNilce M.M.(2004). Electrophoretic molecular karyotype of the dermatophyte*Trichophytonrubrum.Genet. Mol. Biol*. **27** (1):199-102.
- Elavarashi, E.; Kindo, A. J. and Kalyani, J. (2013). Optimization of PCR RFLP directly from the skins and nails in Cases of Dermatophytosis, Targeting the ITS and the 18S Ribosomal DNA regions. J ClinDiagn Res, 7(4): 646–651.
- Evans, E.G.V and Richardson, M.D. (1989). Medical mycology. A practical approach. IRL Press. Oxford University Press. 1st published.

- Gilliand S.E. (1990). Health and nutritional benefits from lactic acid bacteria. *J.FEMS Microbiology Letters.***87**:175-188.
- Ghojoghi, A.; Falahati, M.; Paghehm A.S.; Abastabar, M.; Ghasemi, Z.; Ansari, S.; Farahyar, S. and Roudbary, M. (2015). Molecular Identification of Epidemiological Aspect of Dermatophytosis in Tehran, Iran. J. Research in Molecular Medicine, 3:11-16.
- Goyal, R.; Dhingra, H.; Bajpai, P. and Joshi, N. (2012). Characterization of the Lactobacillus isolated from different curd samples. African Journal of Biotechnology, 11(79): 14448-14452.
- Gräser, Y.; El Fari, M.; Vilgalys, R.; Kuijpers, A. F.; De Hoog, G. S.; Presber, W. and Tietz, H. (1999). Phylogeny and taxonomy of the family Arthrodermataceae (dermatophytes) using sequence analysis of the ribosomal ITS region.*J. Med Mycol***37**:105–114
- Grossi, G.; Mondini, L.; Bruno, W. and Stella, S. (1988).Urinary Phenylacetic Acid Determination by HPLC. *J. Chromotographia* .**26:**142-144.
- Guo, J.; Brosnan, B.; Furey, A.; Arendt, E.K.; Murphy,P. and Coffey, A .(2012). Antifungal activity of *Lactobacillus* against *Microsporumcanis*, *Microsporumgypseum* and *Epidermophytonfloccos um.J.Bioeng Bugs*.**3**(2): 102–111.
- Guo,J.; Mauch, A.; Galle, S.; Murphy, P.; Arendt, E.K. and Coffey, A. (2011) Inhibition of growth of *Trichophytontonsurans* by *Lactobacillus reuteri*. *Journal of Applied Microbiology.* **111**: 474–483.
- Hoque, M.Z.; Akter, F.; Hossain, K.M; Rahman, M.S.M.; Billah ,M.M.and. Islam, K.M.D. (2010). Isolation, Identification and Analysis of Probiotic Properties of *Lactobacillus Spp.* From Selective Regional Yoghurts. *World Journal of Dairy & Food Sciences***5** (1): 39-46.
- Jackson,C.J; Barton, R.C.andEvans,E.G.V.(1999).Species Identification and Strain Differentiation of Dermatophyte Fungi by analysis of Ribosomal-DNA Intragenic Spacer Region. *Journal of Clinical Microbiology*.**37** (4): 931-936.
- Jvo S. (2010). Probiotics. Microbiology Focus. Vol.2.3
- Kane, J.; Summerbell, R.C.; Sigler, L.; Krajden, S. and Land G. (1997). Laboratory handbook of dermatophytes. Belmont CA, USA: Star Publishers.
- Lavermicocca, P.; Valerio, F.; Evidente, A.; Lazzaroni, S.; Corsetti, A.; and Gobetti, M. (2000). Purification and characterization of novel antifungal compounds from the sourdough Lactobacillus plantarum strain 21B. *Applied and Environmental Microbiology*.**66**: 4084–4090.
- Li, H.C.; Bouchara, J.; Hsu, M.M.; Barton, R.; Shuli, S. and Chang, T.C. (2008). Identification of dermatophytes by sequence analysis of the rRNAgene internal transcribed spacer regions. *J Med Microbiol*. **57**(5): 592-600.
- Magnusson, J. (2003). Antifungal activity of lactic acid bacteria. PhD thesis, Agraria 397, Swedish University of Agricultural Sciences, Uppsala, Sweden.
- Mochizuki, T.; Ishizaki, H.; Barton, R.C.; Moore, M.K.; Jackson, C.J.; Kelly, S.L. and Evans, G.V.(2003). Restriction Fragment Length Polymorphism Analysis of Ribosomal DNA Intergenic Regions Is Useful for Differentiating strains of *Trichophytonmentagrophytes*. Journal of Clinical Microbiology, 41(10): 4538-4588.
- Nakanishi, K.; Tokuda, H.; Ando,T.; Yajima, M.; Nakajima, T.; Tanaka, O. and Ohmomo, S.(2002) Screening of lactic acid bacteria having the ability to produce reuterin. Japanese.*Journal of Lactic Acid Bacteria***13**: 37-45.

- Pelinescui, D.; Sasarmani, E.; Chifiriuc, M.; Stoica, I.; Nohit, A.; Avram, I.; Serbancea, F. and Dimov, T.V. (2009) .Isolation and identification of some *Lactobacillus and Enterococcus* strains by a polyphasic taxonomical approach. *J.Romanian Biotechnological Letters*.**14** (2): 4225-4233.
- Piper, C.; Cotter, P.D.; Ross R.P. and Hill, C. (2009) Discovery of medically significant lantibiotics. *Curr Drug DiscovTechnol***6**: 1–18.
- Pyar, H. and Peh, K.K. (2014). Characterization and Identification of Lactobacillus acidophilus Using Biologic Rapid Identification System. International Journal of Pharmacy and Pharmaceutical Sciences, 6(1): 189-193.
- Refai, M.; Abo El-Yazid, H. and El-hariri, M. (2013). Monograph on Dermatophyte. Guide for isolation and identification of dermatophytes, diseases and treatment.Department of Microbiology. Faculty of Veterinary Medicine. Cairo University.
- Renuka, G.; Harish, D.; Pratima, B and NavneetJ. (2012). Characterization of the *Lactobacillus* isolated from different curd samples. *African Journal of Biotechnology***11**(79): 14448-14452.
- Ryan, L.A.M; Dal Bello; F. and Arendt, E.K. (2008). The use of sourdough fermented by antifungal LAB to reduce the125: 274–278.
- Schillinger, U. and Lücke, F.K. (1989). Antibacterial activity of *Lactobacillus* sake isolated from meat. *Appl. Environ. Microbiol*.**55**: 1901-1906.
- Schütz, H. and Radler, F. (1984). Anaerobic reduction of glycerol to propanediol-1,3 by *L. brevis*and *L. buchneri*. *J.Systematic and Applied Microbiology*. **5**: 169-178.
- Siegrist, J. (2010). Probiotics. Microbiology Focus.**2.3**
- Soon, S.H.T and Adam, A.B. (1978).The isolation of dermatophytes at the University of Malaysian. *Malaysian J.***1**:85-89
- White, T.J.; Bruns, T.; Lee, S.and Taylor, J. (1990). Amplication and Direct Sequencing of Fungal Ribosomal RNA genes for Phylogenetics. PCR Protocol and Application. A Laboratory Manual, Publisher: Academic Press, pp.315-322.
- Zourari, A.; Accolas, J. P. and Desmazeaud, H. J. (1992). Metabolism and biochemical characteristics of yoghurt bacteria. J Lait, 7:21-34.

Figures:

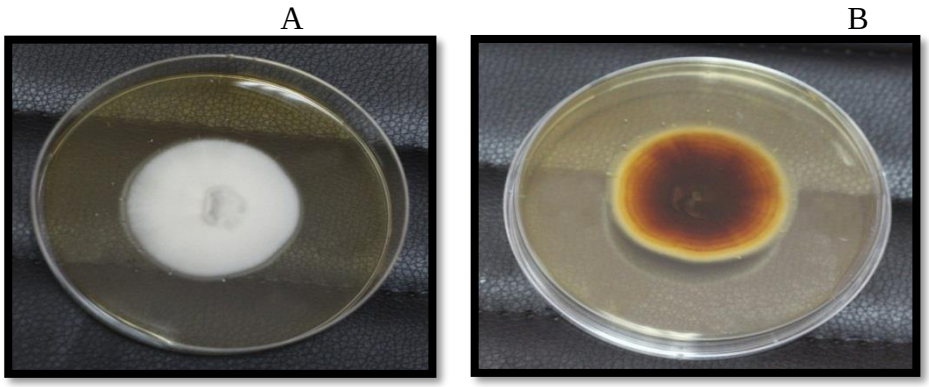

Figure (1): Colonial morphology of *T.rubrum* (downy type) on SDACC at 25°C after two weeks of incubation. A) Colony surface. B) Colony reverse.

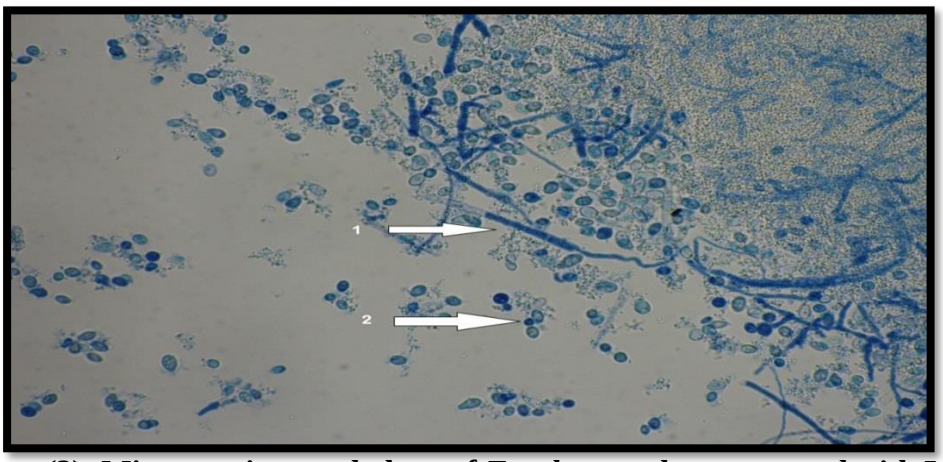

Figure (2): Microscopic morphology of *T. rubrum* colony mounted with LPCB stain showing: 1) Cylindrical shaped macroconidia with appendages. 2) Oval shaped microconidia (40X).

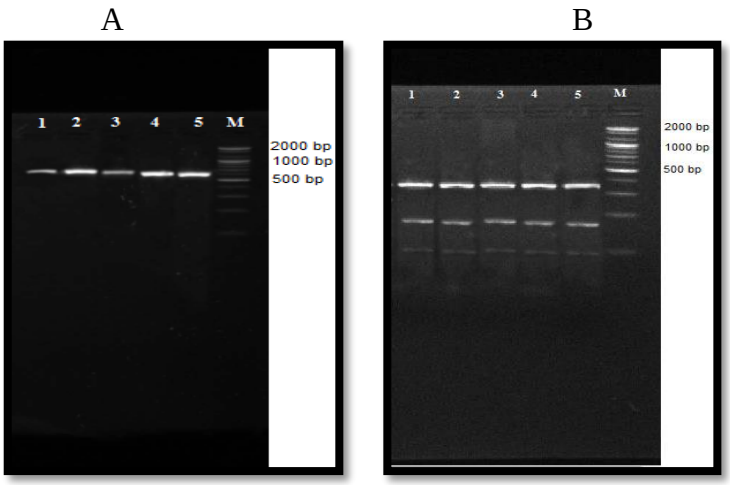

Figure (3): (A) PCR amplified of ITS region in *T.rubrum* 690 bp. B) PCR-RFLP of ITS region in *T.rubrum*380, 180 and 100bp

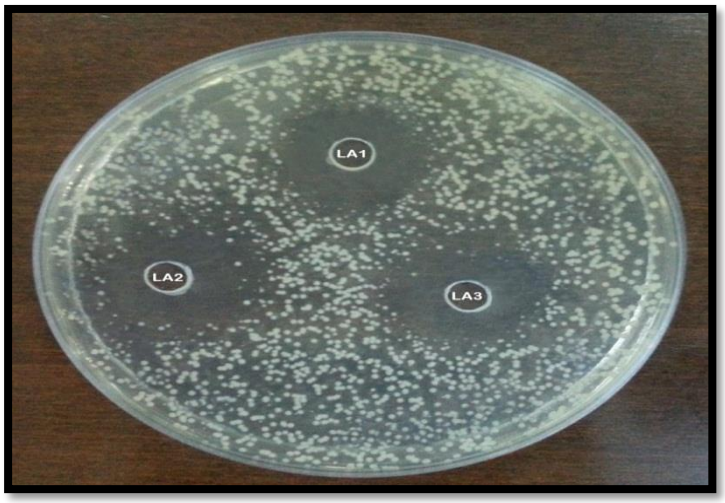

Figure (4): Antagonistic effect of CFS of *L. pentosus (La1)*, *L. plantarum (La2)* and *L. brevis (La3)* on *T. rubrum*.

A B

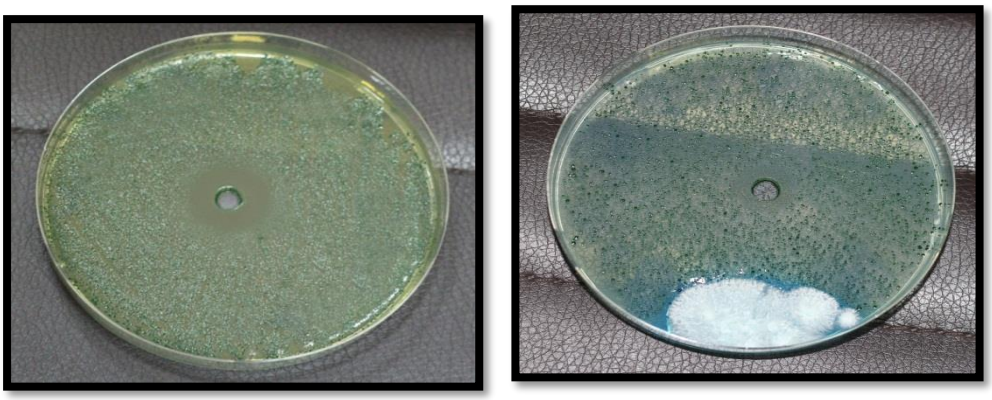

Figure (5): Antagonistic effect of entire cells of *L. brevis (A)* and *L. plantarum (B)* on *T. rubrum*.

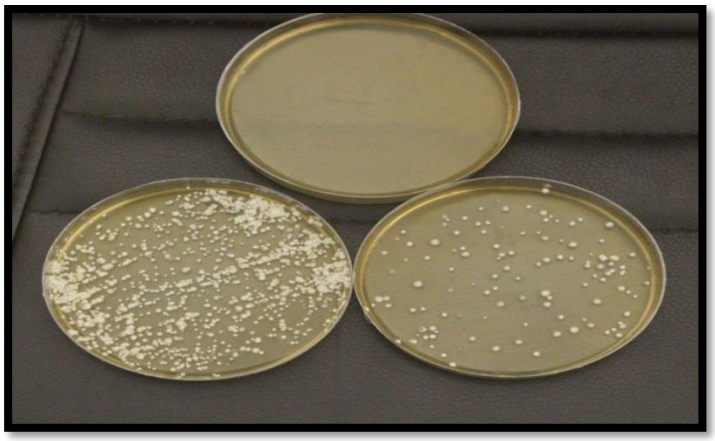

Figure (6): MIC and MFC of *L. collinoidis A*.as entire cells on *T. rubrum*

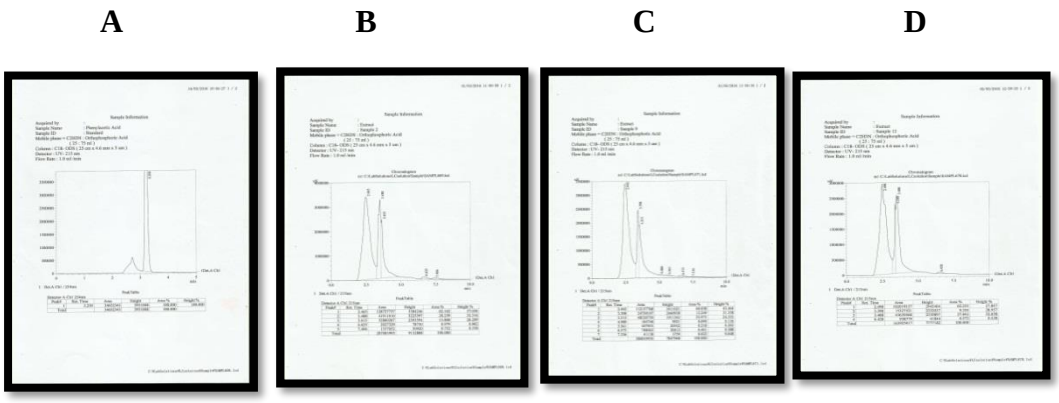

Figure (7): Chromatogram *Lactobacillus spp.* extracted. A) Phenylacetic Acid (PLA) standard. B) PLA in *L. pentosus.* C) PLA in *L. plantarum*. C) PLA in *L. brevis*

Tables:

Table (1): Sugar fermentation from *Lactobacillus spp.* by API 50 CHL kit

N. of isolate	*Lactobacillus spp.*	Source	CTRL	GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO
La1	*L.pentosus*	Stool infant	–	v	–	–	+	+	+	–	–
La2	*L.plantarum*	Vaginal woman	–	-	–	–	V	-	-	–	–
La3	*L.brevis*	Stool infant	–	-	–	–	V	V	V	–	–

N. of isolate	*Lactobacillus spp.*	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN
La1	*L.pentosus*	–	+	+	+	+	–	V	–	–	+
La2	*L.plantarum*	–	+	+	+	+	–	–	–	–	+
La3	*L.brevis*	–	+	+	+	+	–	–	–	–	V

N. of isolate	*Lactobacillus spp.*	SOR	MDM	MDG	NAG	AMY	ARB	ESC	SAL	CEL	MAL
La1	*L.pentosus*	+	–	V	+	+	+	+	+	+	+
La2	*L.plantarum*	–	–	–	+	+	V	V	V	V	+
La3	*L.brevis*	–	–	–	+	+	+	+	+	+	+

N. of isolate	*Lactobacillus spp.*	LAC	MEL	SAC	TRE	INU	MLZ	RAF	ADM	GLYG	XLT
La1	*L.pentosus*	+	+	+	+	–	–	V	+	+	+
La2	*L.plantarum*	+	–	–	–	–	–	–	–	–	–
La3	*L.brevis*	V	V	+	V	V	–	V	–	–	–

N. of isolate	*Lactobacillus spp.*	GEN	TUR	LYX	TAG	DFUC	LFUC	DARL	LARL	GNT	2KG	5KG
La 1	*L.pentosus*	+	V	–	–	–	–	–	–	V	–	–
La 2	*L.plantarum*	+	–	–	–	–	–	–	–	–	–	–
La 3	*L.brevis*	+	–	–	–	–	–	V	–	+	–	–

Table (2): MIC and MFC of CFS of *Lactobacillus spp.* on *T. rubrum*

Lactobacillus spp.	**MIC µ\ ml**	**MFC µ\ml**
Positive Control	125	250
L.plantarum	125	250
L.brevis	125	250
L.pentosus	250	500