

ANTIFUNGAL EFFECT OF *LACTOBACILLUS ACIDOPHILUS* CRUDE EXTRACT

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Adel H. Alwan, Afrah F. Abdulkareem, Baydaa H. Alwan, Antifungal Effect of *Lactobacillus acidophilus* crude extract-Palarch's Journal Of Archaeology Of Egypt/Egyptology 17(7), ISSN 1567-214x

Abstract

This study were carried out to investigate the effect of *Lactobacillus acidophilus* crude extract which produce many active compounds such as organic acids, polyols, exopolysaccharides and antimicrobial compounds, (which have several applications in the food industry) on two types of fungi, including *Candida albicans* , which is isolated from some pregnant women in teaching Bahgdad hospital / Iraq with chronic urinary tract infection, and *Trichophyton rubrum* which is isolated from the central public health laboratory from patients who contracted skin infections. After isolating the fungus diagnosed by phenotypically and microscopically to adopt in the current study. The results showed the strong effect of the bacterial secretions against all the fungi adopted in this study, through the emergence of large inhibition areas in the cultivars planted with fungus after comparing them the size of the mold or yeast under controlling as reached the largest inhibition zone as 25.0 ± 1.7 for to *Trichophyton rubrum*, while the inhibition was complete for *C. albicans* .

Keywords: *Lactobacillus acidophilus*, dermatophytic fungus, *Candida albicans* , urinary tract infection.

Introduction

Fungi are microorganisms that broadly found in nature. They are present in a human body as normal flora in the intestinal system, mouth etc. Hospital-acquired fungal infections may cause dangerous morbidity and mortality, so the fungal diseases are one of the important public health problems. [1]

Trichophyton is a dermatophytic fungus, which is often responsible for nail, hair follicle, and superficial skin infections [2]. In rare cases, it can cause a dermatophyte abscess in deeper skin layers or even disseminate to internal organs, including the lymph nodes, brain, liver, muscle, and bone. This can happen particularly in human immunodeficiency virus-infected or other immunocompromised patients [3]. The deep lesions are usually accompanied by superficial dermatophytosis. *Candida albicans* is a natural flora in the bodies of living organisms, as it can be found in different parts of the human body, such as the digestive system and the vagina. It is considered non-pathogenic, except in some cases of immune weakness and some chronic diseases. [4] As their colonies

are distinguished by their white color when they are grown in agricultural media. (also known as

candidiasis) are common in women, especially when pregnant, this may let the yeast overgrow. [5] Most yeast infections during pregnancy result from the fungus *candida albicans*. Two other kinds of yeast that can also cause them are *candida glabrata* and *candida tropicali*. [6]

Lactobacillales is one of the diverse and phylogenetically heterogeneous orders of lactic acid producing bacteria that include the type genus *Lactobacillus*. *Lactobacillus* spp. are facultatively anaerobic, catalase-negative, Gram-positive, non-spore-forming rods that often grow better under microaerophilic conditions. *Lactobacillus* species most often as an intestinal probiotic capable of eliciting beneficial effects on the microbiota of the gastrointestinal tract. [7]

The species *acidophilus* (meaning acid loving) was perhaps so named because, historically, lactobacilli are isolated from the gastro tract and vagina of humans, where the environment can be quite acidic. [8] Morphologically, *Lb. acidophilus* bacteria are Gram- positive, non-spore-forming rods with rounded ends that occur singly, in pairs, and in short chains. [9] The *Lb. acidophilus* group is fastidious organisms adapted to growth on complex organic substrates and contains mainly obligate homofermentative lactobacilli, but a few are facultative heterofermenters. Lactic acid bacteria (LAB) have been reported to produce antimicrobial substances that inhibit growth of pathogenic and saprophytic microorganisms. [10] Other compounds like organic acids, hydrogen peroxide also included in their antimicrobial effects. Recent many studies concerning on the effects of *Lactobacillus* strains in pathogenic fungi. [11] Focused on treatment and prevention of uro-genital candidiasis and fungal food contamination. Resistance of *Candida* species to different antifungal agents is increasing especially in hospital acquired infections. Despite the established new antifungal agents, it is very important to study antifungal effect of probiotics like *Lactobacillus*. [12]

The aim of the present study: is to find out the effect of bacterial *Lactobacillus acidophilus* extract against some pathogenic fungi isolated clinically.

Material and Methods

Lactobacillus acidophilus: samples were collected from the feces of children who ranged between (1 month - 12 months), where vaginal swabs were taken from uninfected women and cow's milk and cow yogurt from 32 samples. 9 samples of *Lactobacillus* spp. Biochemical tests such as Gram stain, catalase, oxidase, indole production and motion study, were studied to diagnosed the bacteria [13] . After careful diagnosis for all the strains, the *acidophilus* species was chosen to test its effectiveness on some fungal pathogens isolated clinically, because it have antimicrobial substances that inhibit growth of pathogenic and saprophytic microorganisms. [14]

Vaginal swabs were grown directly on Man, Rogosa and Sharpe agar (MRS agar), the other swabs were tested in 50 ml of Man Rogosa Sharpe (MRS broth) after incubation of the culture under optimum conditions (37 ° C for 24 hours) in microaerophilic. Using two MRS agar plates, the first was aerobically incubated and the other anaerobically incubated at 37 ° C for 24, 48 hours Depending on some phenotypic and microscopic characteristics, and biochemical tests in determining the genus of *Lactobacillus*. [15]

Culture of Clinical Specimens:

Isolation of *Trichophyton rubrum*

when using KOH to Direct microscopic examination for the fungus isolated from the central public health laboratory confirmed that it is of the genus *Trichophyton rubrum* then the isolated was cultivated on , dextrose agar plates and potato dextrose agar plates, supplemented with cyclohexamide (500 mg / liter) and chloramphenicol (50 ml / l) to the cultur media , then incubated for 2 weeks at 25-30 ° C. [16]

Isolation of *Candida albicans*

During a period of 3 months, 40 clinically urine samples were collected from pregnant women hospitalized patients and outpatients, who were suspected of UTI in Teaching Bahgdad hospital in Iraq. The results of the initial isolated of the samples that 23 samples were distributed between each of the strains *Pseudomonas aeruginosa*, *E.coli*, *Staphylococcus aureas*, while 17 samples 10 µL of each urine sample was inoculated on blood agar and sabouraud dextrose agar plates.for the initial identification of *Candida* spp. [17]

In addition, *Candida albicans* were diagnosed differentially based on the inoculated in Sabouraud broth supplemented with sodium chloride and incubated at 28 °C. The cultures were visually examined for growth every 24 h. Growth indicates the isolate is *C. albicans*, while no growth after 96 h of incubation indicates the isolate is not *C. albicans*. [18]

Cell Free Supernatant (CFS): On MRS agar *Lactobacillus* was grown and then inoculated in 250 mL of MRS broth with the addition of 5 mL of glycerol and after incubation of the culture at 37 ° C for 24 hours to prepare a bacterial precipitate at a final concentration of 1×10^8 CFU. Through a process of centrifugation at 7200 g for 10 minutes, the bacterial cells are removed, and then the filtration is made using a 0.45 µm mP filter [19]

The Antagonistic test:

CFS method: On the (MHA) plate, 100 µl of the diluted fungal suspension (10^6 CFU) was transferred and spread with a sterile swab and then left for 30 min as triplicate. The surface of the MHA plate was perforated by a pasture pipette, three wells of 6 mm in diameter and filled with 60 µl of CFS from each *Lactobacillus* spp. broth. At 25 ° C for 18-24 hours all dishes were incubated [19]. Zone of damping around the well showed a positive result. The experiment was repeated three times and the area of inhibition was averaged with \pm standard deviation and compared with the control group (CFS of MRS broth).

Results and Discussion

Clinically isolate for *T. rubrum* diagnosed based on macroscopic morphology which characterized by white colonies at the surface and became creamy, suede like to downy microscopically, there are numerous oval shape microconidia and cylindrical. (Figure 1) [20]

The role of immune system status during pregnancy in organism pathogenicity remains controversial. so as prevalence of infection increases with age and lower socioeconomic grouping. Concomitant urinary tract anomalies and maternal disease (for example, diabetes or sickle cell disease) also significantly increase

risk. In present study 40 randomly urine samples were collected from pregnant women. [21] To find out the common microbial causes in pregnant women, see the cause of UTI, as the results of this study showed the diversity of infection in pregnant women, where the results varied between 11 (27.5%) for *E.coli*, 7 (17.5%) for *Pseudomonas aeruginosa* and 5 (12.5) for *Staphylococcus aureas* respectively , depending on microscopical examination and formation of germ tube as available for rapid identification of yeast., as the test results showed that highest rate of infection was due to yeast *Candida albicans*, when a rate of infection of 17 was recorded, which is equivalent (42.5 %) to the total samples of strains under study as shown in the (Table1)

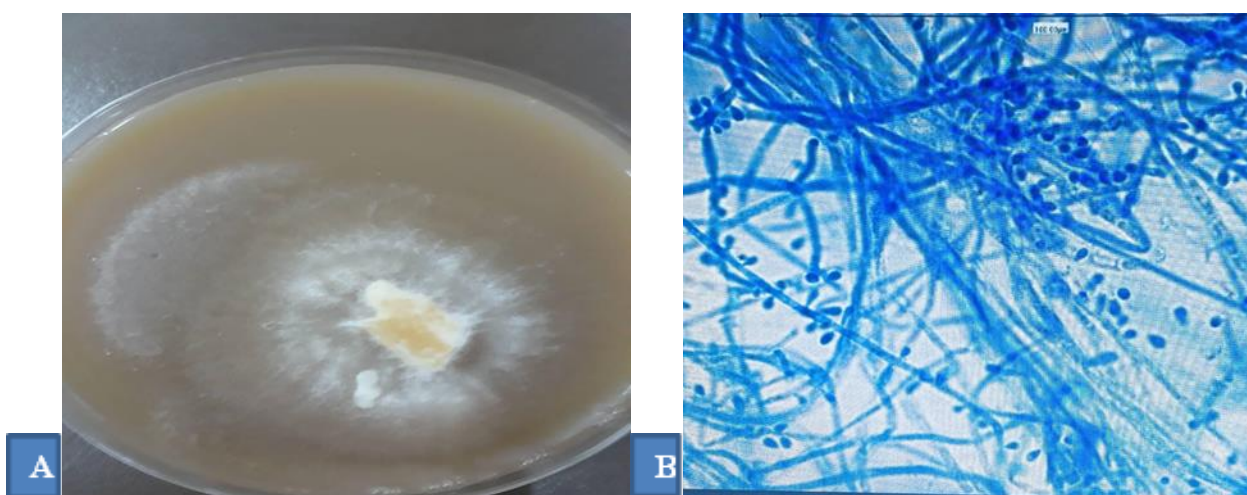


Figure (1): A. White colonies of *T. rubrum* (downy type), B. microscopically shape

this result it is agree with many other studies on specifically candiduria, *Candida albicans* is shown to share 50-70% burden followed by *C. galabrata*, and *C. tropicalis*, which is the third most common species [22,23]. Similar findings were recorded in this study also except presence of another genres of bacteria of antibiotic resistance [24]

Microscopic Examination showed rod to cocci shaped bacteria, gram positive, none spore forming and produced small size, spherical. on the surface of MRS agar medium all isolates produced white to creamy color colonies . Biochemical tests, such as fermentation of carbohydrates, aerobic growth reaction, motility, growth at the fixed temperature, and gas production from glucose, were performed by the methods of Mitsuoaka [25], by using API 20. isolates were assigned as *Lactobacillus*

Anti-fungal bacterial activity: The present study focused on Anti-fungal bacterial activity of *Lactobacillus acidophilus* against *T. rubrum* and *C. albicans* .The study was performed using the MHA and MRS agar , surface well agar diffusion , CFS and *T. rubrum* were incubated at 25 ° C. The results showed that the zone of inhibition reached 25.0 ± 1.7 compared with the fungus growth without treatment under the same conditions at 19.3 ± 1.7 mm . While the inhibition was complete for *C. albicans* , as from these results, the strong effect of bacterial extract against *T. rubrum* and *Candida albicans* results are consistent with [26,27] he found in his study, Similar[28] increased inhibitory activity in the year 2012 Gerbaldo *et al.*

The production of hydrogen peroxide, bacteriocin, and lactic acid from lactobacilli as secondary metabolites may be the active antifungal substances.[29]. Similar studies indicated which *Lactobacillus delbruecki*, *L. alimentarius*, *Lactobacillus fermentum*, *Lactobacillus delbruecki* was observed they had antifungal effects.

CONCLUSION: Dietary supplementation with *L. acidophilus* could alleviate the inflammation and fungus infections, and According to the results, by comparing the results, we can say that the Bacterial Cell Free Supernatant (CFS), as probiotic of lactobacilli defense mechanisms against bacterial and fungal pathogens due to the favorable effects of antimicrobial effects, and these bacteria, participate most in the food industry and can be used to further the health of as natural products without any side effects.

Table 1: Types of Microbial Infections in Urine of Some Pregnant Women

Isolates	The percentage of infection (%)
<i>Candida albicans</i>	17 (42.5)
<i>E.coli</i>	11(27.5)
<i>Pseudomonas aeruginosa</i>	7(17.5)
<i>Staphylococcus aureas</i>	5(11.5)

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