Lactobacillus Rhamnosus GG Role in the Suppression of Candida Albicans Causing Candidiasis (Thrush)

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Abstract

Introduction: It is important to distinguish infecting strains of Candida albicans because isolates of Candida species vary widely, both in their infection-causing capacity and in their sensitivity to antifungal agents. Material and method: Thus this study provides isolate candida and identification of isolates from the oral cavity and test the antimicrobial activity of *Lactobacillus rhamnosus GG* and their supernatants on the viability of *Candida albicans* isolates. The diagnosis was based on conventional methods, and genetic diagnosis was confirmed by PLC1 gene (PhosphoLipase C) amplification, the design primers were done by using the database of Bank of NCBI-Gene and online design, all isolates gives positive PCR products with molecular weight 459bp,

Result: The results showed that *Lactobacillus rhamnosus GG* and their supernatants decreased the log cycles growth of all *Candida albicans* isolates with average (1.7 -2.1) logarithmic cycle for live cells and (1.28 -1.79) logarithmic cycle for *Lactobacillus rhamnosus GG* supernatants, **Conclusion**: *Lactobacillus rhamnosus GG* may be a suitable and effective alternative to treatment oral candidiasis free from side effects.

Keywords: *Oral candidiasis, thrush, C. albicans, Lactobacillus rhamnosus GG*

Introduction

Candidiasis of Oral is the oral cavity opportunistic infection. It is common, associated with microbial imbalance in the mouth among the elderly, especially in the wearing of dentures and newborns. It can be facilities for some diseases, such as diabetes and immunodeficiency[1]. Oral candidiasis is caused by excessive growth of candida, most notably *C. albicans*, which account for more than 80%. In humans, candidiasis is the most common fungal disease. In particular, in newborns and older people, the rates ranging from 20- 75% without any symptoms, 45-65% of healthy children, 30-45% of healthy adults, 50-65% of individuals with missing teeth, 65-88% of those in long-term hospitals, 90% of cases of acute leukemia undergoing chemotherapy, and 95% of patients with HIV[2]. *C.albicans* is a natural oral flora and does not cause any problems in healthy people, but overgrowth can lead to infection, Their development, however, is typically restricted by the human immune system and by competition from other microorganisms, such as mouth-occupying bacteria. White spots on the tongue or other parts of the mouth and throat are symptoms and indicators.Other symptoms may include soreness and problems swallowing, the newly proposed classification includes primary oral candidiasis, where the condition is confined to mouth and tissue around the mouth, and secondary oral candidiasis, where other parts of the body, as well as the mouth, are involved, the HIV / AIDS pandemic was an important factor in leaving the traditional classification because it a new community of patients with atypical types of oral candidiasis 3 resulted in the formation of. Three major clinical manifestations of Candida are commonly known: pseudomembranous, erythematous (atrophic) and hyperplastic [4]. Sometimes there can be more than one clinical variable in the same person [5]. When tested in vitro, *C. albicans* is typically susceptible to all widely used antifungals. [6]*Lactobacillus rhamnosus* GG possesses the ability in antifungal

strategies, especially against *C. albicans*, as studies have shown their ability to inhibit growth, formation of hypha and adhesion [7]. Also, *Lactobacillus rhamnosus* GG have inhibitory activity to destroy the main polymer of the hypha cell wall, chitin. Thus selecting to be used as potential probiotics in patients with *C.albicans*

Material and Methods

Samples were taken from the tongue using cotton Pellet and saliva using cotton roll. Under the supervision of a dentist in sterile conditions, the samples were transferred to sterile physiological solution and mixed with Vortex (Griffin- England) for one minute, samples were streaked on Sabouraud dextrose agar plates (SDA) incubated with modified Chloramphenicol (0.05 mg/L) to 37 C 8 for 48-72 hours. Isolates with a creamy to yellowish colonies color, smooth at 30ºC and positive result of formation germ tubes in germ tube test were accepted and considered as positive specimens for *Candida albicans*[9]. Isolates genomic was

by means of Spin Column Fungal Genomic DNA Mini- Preps Kit (EZ-10) and carried out according to instructions of (BioBasic, Canada) company .The purity and quantity of extracted DNA was estimated by using the Nanodrop spectrophotometer (THERMO. USA). PCR technique was performed for detection important virulence factor genes in*Candida albicans*. according to [10]. The primers specific for PLC1 gene (PhosphoLipase C) were designed by the means of online database of NCBI-Gene Bank and supported from Bioneer Company, South Korea. The forward sequence 5 '-CCTGTTAGCACCCCCTTGTT-3 "and reverse Sequence 5"- AACACATCGACACCCACGTT-3. PCR thermocycler conditions done using serial PCR thermocycler system as initial denaturation at 95ºC for 5 min, denaturation at 95ºC for 30 sec annealing 58ºC for 30 secs, time for extension 45sec at 72ºC and final extension 72ºC for 7 min. All these steps repeated 30 cycles. PCR products were examined on agarose gel (1%) using horizontal electrophoresis unit (Bioneer, Korea) at 5v/cm for 2 hours after ethidium bromide staining, then DNA bands were visualized by using U.V transilluminator (Vilber-Lourmat, France) at 365 nm.

Preparation of *Lactobacillus rhamnosus GG* culture supernatants

Pure cultures of *Lactobacillus rhamnosus* GG was obtained kindly from Food Science and Biotechnology Department, College of Agriculture, Baghdad University, *Lactobacillus rhamnosus* GG was grown and kept in MRS medium (Himedia, India) and activated in skim milk 12% , incubated at 37 °C for 24 h, Cells were harvested subsequently the incubation period by centrifuged at 10,000 rpm for 5 min, then the supernatant was changed to pH 5.5±1 with 1N NaOH to eliminate the effect of putative organic acids produced then filtered with Millipore 0.22 µm pore size (Pall, USA)[11].

Antimicrobial Activity of *Lactobacillus rhamnosus* GG culture and their supernatant against *C. albicans*

The antibacterial actions of *Lactobacillus rhamnosus* GG supernatant Opposed to *C. albicans* were assessed according to [12] with some modifications. 250 µl of a *C. albicans* suspension and 250 µl of a *Lactobacillus rhamnosus* GG suspension □10^7 CFU/mL (or culture filtrate) were mixed with 1.5 ml of BHI broth. PBS with *Lactobacillus rhamnosus* GG suspension was used as control, cultures wereincubated at 37°C for 24 h (5% CO_2). After incubation, the cultures were diluted and plated on Sabouraud dextrose agar (Difco) supplemented with chloramphenicol (0.05 mg/L). The plates were incubated at 37°C for 48 hs. *C. albicans* was evaluated as a logarithm of viable numbers by Pour plate count method.

Ethical aspects

Informed written consent of the Ministry of Health in Iraq, was obtained from all patients that accepted to participate in this study.

Results & Discussion

From the total that collected nine samples gave colonies appeared cream to white color and smooth at 30 ºC. also positive result of formation of germ tubes in germ tube test [15].

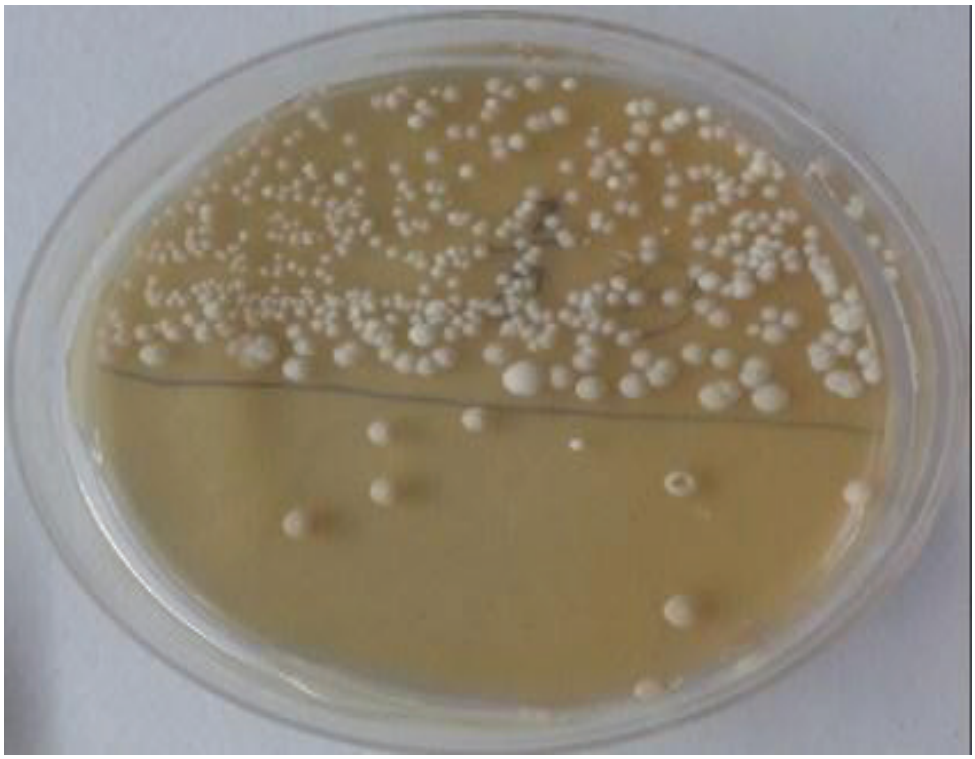

Figure (1): *C.albicans* growth on SDA

The presence and integrity of the extracted DNA from nine isolates of Candida albicans was confirmed by agarose gel electrophoresis, as shown in the figure (1).

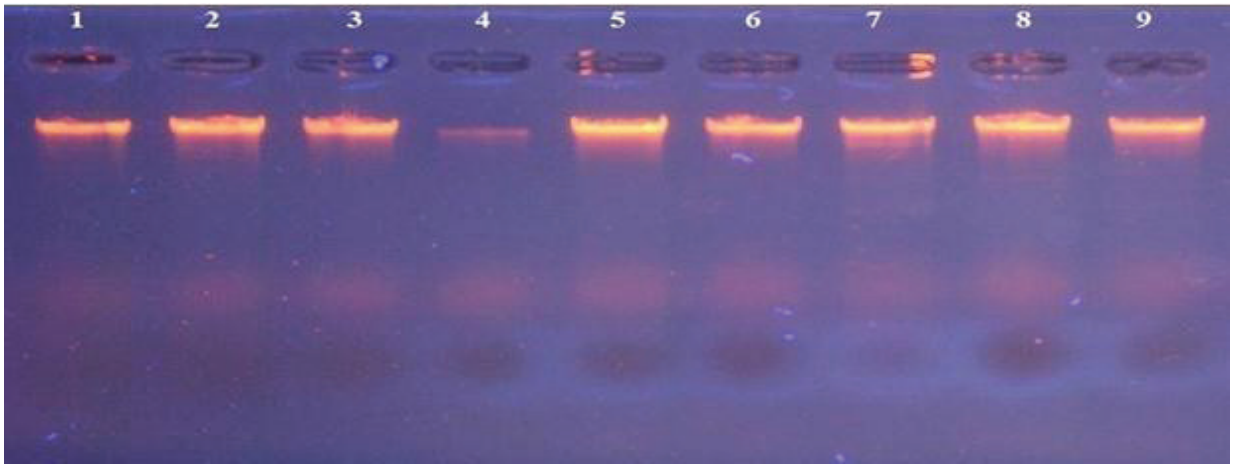

Figure (2): Gel electrophoresis for *Candida albicans* DNA bands for positive isolates. Lane (1-9) positive isolates Lane control.

The amplification result was executed on the extracted from of DNA for all studied specimens and established by analysis of electrophoresis. By this analysis, the DNA strands produced from the successful bindingbetween target gene (PLC1) specific primers and extracted DNA specimen. Under UV. Light and by using the ethidium bromide as specific DNA stain, the successful binding appeared as single compact bands. Depending on DNA marker (1500-100bp DNA ladder),the electrophoresis was used to estimate the molecular size of DNA and the estimation result exposed that the PCR product (amplified DNA) was 459bp for C. albicans showed as figure (2).

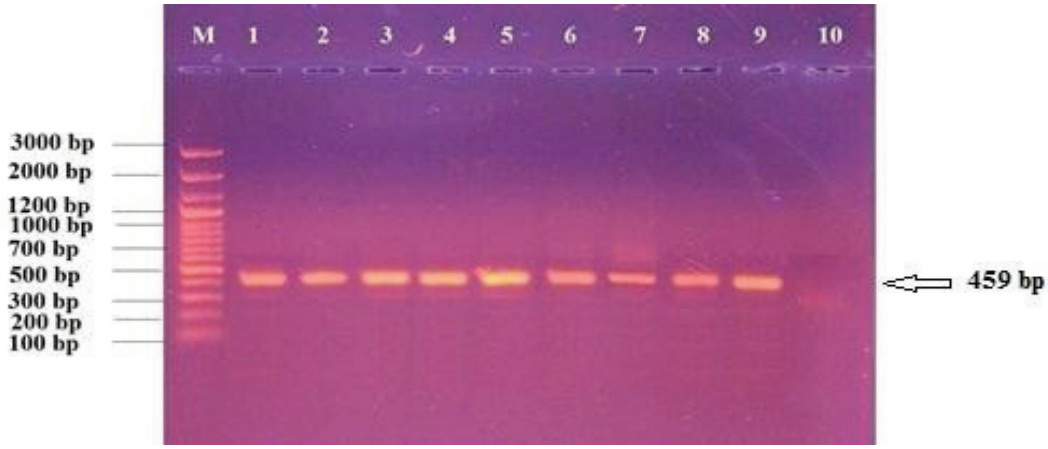

Figure (3): Gel electrophoresis image for PLc1 gene (Virulence factor gene) in Candida albicans isolates. electrophoresis was achieved on 1% agarose gel and run with a 5v/cm present for 120min. Lane M: markers Lane (1-9) PCR products amplified from 9 isolates,Lane 10 negative samplecontrol.

PLc1 gene is virulence factors of *Candida albicans* encode phospholipases with conserved phospholipase and also known to be important regulators of cellular processes causes hydrolysis of phosphatidylinositol 4,5-bisphosphate [13]. PLC1 has been shown to be transcribed in hyphae forms of *Candida albicans*, encodes a large protein containing 1099 amino acids with X and Y domains [14]. The diagnosis of *Candida albicans* is considered a great importance to differentiate between the strains because some of them are of significance pathogenicity and their difference in their ability pathogenicity and Virulence factors [8].

Antimicrobial Activity of *Lactobacillus rhamnosus GG* and their supernatants on the viability of *Candida albicans* isolates

Results showed the antimicrobial activity of *Lactobacillus rhamnosus GG* and their supernatants on the viability of *Candida albicans* isolates (Figure 3). Live cells of *Lactobacillus rhamnosus GG* decreased the log cycles of all *Candida albicans* isolates with average (1.7 -2.1)logarithmic

cycle, also *Lactobacillus rhamnosus GG* supernatants decreased the log cycles of all *Candida albicans* isolates with average (1.28 -1.79) logarithmic cycle.

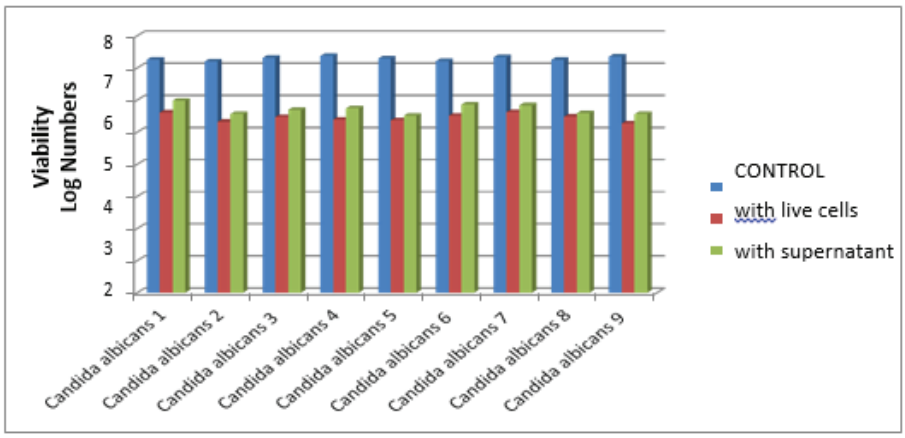

Figure (4): The effect of *Lactobacillus rhamnosus* GG and their supernatants on the viability of *Candida albicans* isolates

Lactobacillus rhamnosus GG clearly suppressed the growth of all *Candida albicans* isolates, *Lactobacillus rhamnosus* GG role is to protect the oral epithelium against *C. albicans*, as it prevents adhesion and colonization, and restore the microbial balance 16.*Lactobacillus rhamnosus* GG inhibits varlenus vactores of *Candida albicans,* prevented from adhesion and the production of inhibitory factors for the formation of hyphae [17].as shown in figure (4)The chance of high Candida counts was decreased by 75 per cent by *Lactobacillus rhamnosus* GG intervention *18*.studies found Significant reduction of *Candida* infection after *Lactobacillus rhamnosus GG* administration, and *Candida albicans* was the most prevalent species before and after the *Lactobacillus rhamnosus GG* therapy [19]. *Lactobacillus rhamnosus GG* protects oral epithelial tissue Furthermore, Additionally, LGG has Inhibit the virulence factors of candida like adhesion to oral epithelia and hyphae [20].

Conclusion

Candida albicans infection have increased significantly in the last few years due to increased application of immunosuppressive therapies.they are a serious problem and have become more resistant to common antibiotics 21. Strains of Antibiotic-resistant were rapidly spread, making these antibiotics ineffective. Therefore, there is currently no effective way to treat C. albicans infection that can develop the infection to become life- threatening sepsis once Candida albicans can enter the bloodstream. In addition, these patients often take antibiotics, some of which have serious side effects (nausea, vomiting, and diarrhea). Lactobacillus rhamnosus GG is able to inhibit the growth of candida albicans and modulate the immune reaction of oral mucosa cells, this encourages their use in oral health field and the preparation of pharmaceutical preparations for thrush treatment.

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Conflict of Interest: That no competing interest exists.

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