

The Inhibitory Effect of *Lactobacillus acidophilus* and *Lactobacillus plantarum* against *Candida albicans* Associated with Denture Stomatitis

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Abstract:

In this study *Candida speices* was diagnosed in 26 swab samples from patients with denture stomatitis , investigates the antagonism activity of *Lactobacillus* was investigated against the yeast of *Candida albicans in vitro*.Results revealed that The inhibition effect of Lactic Acid Bacteria against *C.albicans* was examined in solid medium, *L.plantarum* gave higher inhibition average 11mm followed by *L.acidophilus* with average 9 mm and, *L.fermentum* , *L.casei* with averages 7 mm. Whereas the filtrates, the highest inhibition zone were 20 and 16 mm by *L. plantarum* and *L.acidophilus*, respectively.

Key words: denture stomatits, Lactobacilli, *Candida albicans*.

Introduction:

Lactic Acid Bacteria (LAB) such as *Lactobacillus acidophilus*, are important components of the normal intestinal microflora in humans and animals. The *L. acidophilus* and Bifidobacteria are mainly used as probiotics [1]. Probiotics are organisms or supportive substances that aid in restoring and maintaining a healthy intestinal balance in favor of healthful bacteria ,which is essential in maintaining good health, [2]. The choice of Lactobacilli as probiotic agent is appropriate since the normal gastrointestinal microbiota of man and animals is rich in this organism[3,4]. There are instances where bacteria provide fungi with compounds that enhance the production of fungal virulence determinate. Other bacteria produce factors that are likely to inhibit pathogenesis by repressing fungal filamentation. [5]. *Lactobacilli* as

probiotic organism possess some important properties. These include the ability to adhere to host cells, to exclude or reduce pathogenic bacteria, and to produce acids, hydrogen peroxide, and bacteriocins antagonistic to the growth[2].

Denture stomatitis is an oral pathology of multifactorial etiology that affects a large number of patients using complete or partial dentures. The main etiologic factors related to denture stomatitis are trauma, poor oral hygiene and infection with *Candida species*. In general, the patients with denture stomatitis complain of edema, hyperemia, pain in the affected areas and burning mouth when a low salivary flow is taking place [6].

The aim of the present study is to evaluate the ability of Lactic acid bacterial species to inhibitory *Candida*

albicans associated with denture stomatitis.

Materials and Methods:

Collection of Samples

Twenty six swab samples were collected from patients suffering from denture stomatitis in Baghdad city during the period from January 2013 until June 2013 in The Dentistry college Hospital. The swabs were collected aseptically and transported immediately into test tube containing normal saline (0.9%) to the laboratory [7]. These swabs were cultured on different media for the isolation and identification of *Candida* species.

Yeast isolates

Swabs sample were obtained according to the procedure described by McGinniz [8]. Yeast cells were cultured on Sabouraud Dextrose Agar (Oxoid, USA) supplemented with chloramphenicol, and incubated at 37°C for 72 h, yeast isolates were identified following the scheme of Buckley, *et al* [7] and Ellis [8] Including germ tube test, production of chlamydo spores, biochemical tests, and api-candida [9].

Bacterial isolates

L. acidophilus, *L. casei*, *L. fermentum*, and *L. plantarum*. isolated from healthy oral cavity were cultured in MRS broth or Agar, incubated anaerobically in anaerobic jar at 37°C for 48hrs and identification by biochemical tests and molecular methods (PCR). MRS agar plate was streaked and incubated at 37°C for 24 hours with 5% CO₂ to ensure the purity of the culture. One colony from the MRS agar plate was inoculated in MRS broth for 24 hours at 37°C. A 1% (v/v) inoculum was subcultured into MRS broth and incubated at 37°C for 24 hours for the assays.

The Inhibition of *Candida albicans* by *Lactobacillus* filtrations and live cells

Candida albicans were tested for inhibition by the probiotic filtration and live cultures of the following *Lactobacillus* species: 1) *L. acidophilus*, 2) *L. casei*, 3) *L. fermentum*, 4) *L. plantarum*. A culture of *Lactobacillus* was inoculated in MRS broth then incubated at 37 °C for 24 hr. After that, the culture was streaked on surface of MRS agar then incubated at 37°C for (48) hr. [10].

After incubation, discs were made (5mm). The disc was fixed on the surface of nutrient agar plate culturing with *C. albicans* that is previously spreaded with Lactic acid bacteria, and then incubated at 37°C for 48 hr. After incubation, inhibition zone around the discs was measured.

The supernatant was collected from *L. plantarum* and *L. acidophilus* after inoculating MRS broth alone with 1% of *Lactobacillus* isolate that selected in culture in a test tube, the tube was incubated at 37°C for 48 hr incubation periods. After incubation, the culture was centrifuged at 6000 rpm for 10 min to get the supernatant which contained the filtrate of grown cells. Then it was filtered through Millipore filter 0.22µm unit. Wells diffusion method was used to detect the inhibitory effect of *Lactobacillus* against *C. albicans* by making wells on nutrient agar surface, and filling them with the filtrates of *lactobacillus*. Following 48 hours, the diameters of the inhibition zones were measured for each well.

Statistical Analysis

The Statistical Analysis System-SAS, [11] was used to the study effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Result and Discussion:

In the present study, the antagonism activity between *Lactobacillus* species and pathogenic *C. albicans* was investigated using digging agar method.

The Inhibitory effect of *Lactobacillus* cells

The results showed that most isolates possess inhibitory effect at various levels (Table 1) and (Figure 1). Significant differences at ($P < 0.05$) between the diameters of the inhibition zones of lactic acid bacteria isolates. *L. plantarum* gave higher inhibition average of 11 mm followed *L.acidophilus* with 9mm and *L.fermentum* , *L.casei* with averages 7mm.

Table (1) Inhibitory Effect of *Lactobacillus* isolates against *C.albicans* in Nutrient Agar at 37C for 48hrs

| <i>Lactobacillus</i> spp | Diameter of inhibition zone (mm) |
|--------------------------|----------------------------------|
| <i>L.plantarum</i> | 11.0 ± 0.69 |
| <i>L.acidophilus</i> | 9.0 ± 0.57 |
| <i>L.fermentum</i> | 7.0 ± 0.49 |
| <i>L.casi</i> | 7.0 ± 0.49 |
| LSD value | 2.345* |

* ($P \leq 0.05$).

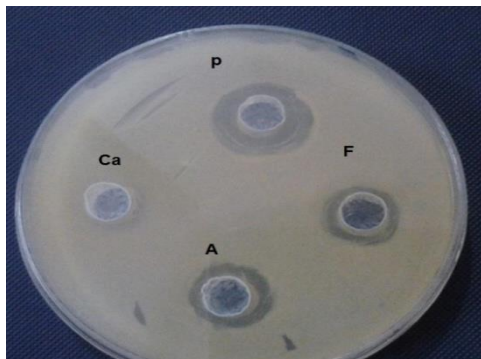


Fig.(1):Inhibitory Effect of *Lactobacillus* isolates against *C.albicans* in Nutrient Agar at 37C for 48hrs

*A:*L.acidophilus*;Ca:*L.casi*;F:*L.fermentum* and P:*L.plntarum*

This may due to production of inhibitory compounds by *Lactobacillus* spp. especially organic acids, and bacteriocins. These results are in agreement with Noverr and Huffnagle [12] who found that the antagonist activity of lactic acid bacteria against *Candida albicans* might be referred to its ability produce organic acids which lower pH, bactriocins, and competition on the nutrients and inhibited germination of *C. albicans*. evidences mentioned suggested that live bacteria of probiotics were effective in preventing candida gut colonization and systemic dissemination. Although many reports proved that lactobacilli and bifidobacterium exerted excellent ability of adhesion to the intestinal mucosa .Hooper and Gordoan, [13] the antagonism of live probiotics to the *C. albicans* appeared due to the production of active metabolites rather than the competitive exclusion.

The Inhibitory effect of *L. acidophilus* and *L.plantarum* filtrate

Well diffusion method has been used to determine the inhibitory effect of *L. acidophilus* and *L. plantarum* filtrates against *Candida albicans*. Results showed that significant differences between ($P < 0.05$) the diameters of the inhibition zones of lactic acid bacteria isolates .the inhibition zone was (20, 16) mm produced by filtrats of *L. plantarum* and *L.cacidophilus* respectively .(Table 2)(Figure 2).

Table(2) Inhibitory effect of bacterial filtrations against *C.albicans* in Nutrient Agar at 37C for 48hrs

| <i>Lactobacillus</i> spp | Diameter of inhibition zone (mm) |
|--------------------------|----------------------------------|
| <i>L.acidophilus</i> | 16± 0.65 |
| <i>L.plantarum</i> | 20± 0.87 |
| LSD value | 2.435 * |

* ($P \leq 0.05$)

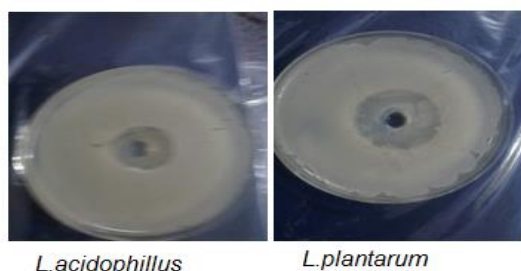


Fig. (2): The Inhibitory effect of bacterial filtrations against *C.albicans* in Nutrient Agar at 37C for 48hrs

These results corroborated with the study conducted by Chikthimmah *et al.*, [12]. Barefoot and Klaenhammer [14] stated that inhibitory substances of lactic bacteria increase upon increasing concentration of its filtrates, and death of pathogenic increases with increasing the inhibitory substances like bacteriocin, of *L.plantarum*.

Noverr and Huffnagle [15] found that short chain fatty acids (butyric acid) the product of Lactic acid bacteria (LAB), inhibited germination of *C. albicans*. In addition LAB culture supernatants as well as live LAB also inhibited *C. albicans* Morphogenesis [16]. Other studies reported that successful inhibition of *C. albicans in vitro* could be due to inhibitory effect of acetic and lactic acids produced by *L. acidophilus*. Other investigators related this anticandidal activity to hydrogen peroxide (H_2O_2) production [17].

Ignacio and Thai [18] found the antifungal properties of Probiotics have been suggested to be due to its lactic acid production and microbial attachment to enterocytes, further studies may evaluate possible antifungal activity at higher concentrations. Due to the limitations of *in vitro* studies and the spectrophotometer method used in evaluating growth rate, the full potential antifungal activity of probiotics could not be tested. Further

studies should be conducted to evaluate the role of the colonization of normal flora and probiotics in prohibiting the over growth of potential harmful organisms such as *Candida*.

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التأثير التثبيطي لـ *Lactobacillus acidophilus* و *Lactobacillus*
ضد *plantarum* المرافقه التهابات اطعم الاسنان
Candida albicans

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الخلاصة:

شخصت خميره *Candida spp* في 26 مسحة لعينات من التهاب اطعم الاسنان. ودرست الفعالية التثبيطية لانواع بكتريا حامض اللبن *Lactobacillus* ضد خميره *Candida albicans*. احدثت البكتريا *L.plantarum* اعلى هاله قطر تثبيط 11ملم ، تبعثها *L.acidophilus* بقطر تثبيط 9ملم ، اما *L.casi* ، *L.fermentum* فقد احدثت تأثيرا متساويا بقطر هاله تثبيط قدرها 7ملم . أما بالنسبة لتأثير الراشح لكل من *L.plantarum* و *L.acidophilus* فقد بلغت أعلى هاله قطر تثبيط 16,20 ملم على التوالي.

الكلمات المفتاحيه: التهابات الاطعم، بكتريا حامض اللبن، خميره الكانديدا