Probiotic effects of feeding live Lactobacillus acidophilus to Candida albicans-colonized oral cavity of rats.

Nida'a S. Hamad Fatima M. Abas Hassanen K. Ibraheem

Department of Biology , College of Science for women.

University of Babylon, Babylon, Iraq

Abstract

This study investigates the antagonism activity of *Lactobacillus acidophilus* against the yeast *Candida albicans in vitro*. Results revealed that *L. acidophilus* with high antagonism activity and inhibited growth of *C. albicans*. Amodel for oral candidiasis in BALB/c rats was established . After inoculation with 1×10^8 *C. albicans* yeasts, these rats displayed increased levels of oral colonization , oral thrush appeared after 4 days of infection . Rats were fed live *L. acidophilus* for assessing its capacity to control the oral infection by elimination the yeast from the oral cavity. Data showed that feeding live *L. acidophilus* for 15 days significantly shortened the duration of oral colonization compared to control animals.

الخلاصة

في هذه الدراسة تم بحث الفعالية التضادية لبكتريا *Lactobacillus acidophilus* ضد خميرة *Candida albicans* . تم عمل نموذج *C.albicans* فعالية تضادية عالية حيث ادت الى تثبيط نمو الـ *L. acidophilus* الزجاج . اظهرت النتائج ان لبكتريا *C.albicans* ، حيث لقحت بـ١× ١٠ ^ ٨ /مل من خلايا BALB/c للأصابة الفموية بالمبيضات في الجرذان السويسرية من سلالة اظهرت الجرذان زيادة في مستوى الاستعمار الفموي اذ ان السلاق الفموي ظهر بعد مرور ٤ يوم من الاصابة . تم تغذية الجرذان لتقيم قدرتها في السيطرة على الاصابة الفموية عن طريق ازالة الخميرة من التجويف الفمي . *L. acidophilus* بخلايا حية لبكتريا لمدة ١٥ يوم كانت ذات تاثير معنوي في تقليل امد الاستعمار *L. acidophilus* اظهرت النتائج ان التغذية بخلايا حية من بكتريا الفموي مقارنة مع حيوانات السيطرة.

Introduction

Mucosal candidiasis is extremely common in immunocompromised patients, the prevalence of site – specific infection (i.e., oropharyngeal, vaginal and esophageal candidiasis) can be quite variable depending on the immune status of the host . The majority of cases of episodes of mucosal candidiasis are caused by Candida albicans , a dimorphic fungal commensal organism of the gastrointestinal and lower female reproductive tracts , as well as being an opportunistic pathogen, capable of causing a wide range of infection [1,2,3].Conversion from the yeast to

the hyphal from has been associated with increased virulence and mucosal invasiveness [4].

Risk factors include impaired salivary gland function , drugs, dentures high carbohydrate diet , and extremes of life, smoking , diabetes mellitus, Cushing's syndrome , malignancies and immunosuppressive conditions [5].

Lactic Acid Bacteria (LAB) such as Lactobacillus acidophilus, L. delbrueckii spp. bulgaricus and Bifidobacteria are important components of the normal intestinal microflora in humans and animals . The L. acidophilus and Bifidobacteria are mainly used as probiotics [6]. 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, and include lactic acid bacteria and bioactive proteins such as immunoglobulin A and Lactoferrin [7].

The choice of Lactobacilli as probiotic agent is appropriate since the normal gastrointestinal microbiota of man and animals is rich in this organism [8,9,10] . There are instances where bacteria provide fungi with compounds that enhance the production of fungal virulence determinates. Other bacteria produce factors that are likely to inhibit pathogenesis by repressing fungal filamentation . [3].

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 of pathogens [11,12,4,13].

This study was performed prospectively to test the antagonistic ability of L. acidophilus against C. albicans on Sabouraud Dextrose Agar (SDA.) and in animal modle , determine the period for elimination of the yeast from oral cavity following feeding live L. acidophilus.

Materials and Methods

Fungal culture

C. albicans was isolated from women suffering from urinary tract infection . Urine sample was obtained according to the procedure described by McGinniz [14].

Yeast cells were cultured on Sabouraud Dextrose Agar (Oxoid, USA) supplemented with chloramphenical, and incubated at 37C° for 72 h , yeast isolate was identified following the scheme of Buckley [15] and Ellis [16] including germ tube test, production of chlamydospores and physiological and biochemical tests, the the yeast cells were cultured in Sabouraud dextrose broth (Oxoid) for 48 h at 25C° in a shaking water bath . The blastospores were transferred into fresh medium and cultured at 25C° for a further 18 h. The blastospores were colleted by centrifugation , washed twice with phosphate-buffered saline (PBS) and then adjusted to a concentration of 1×108 blastospores / ml of PBS according to the procedure described in [17] .

Bacterial isolate

L. acidophilus used in this study was isolated from fermented milk products . Sample was diluted (1:100) and inoculated onto Skim Milk agar (Himedia , India) and incubated at 37C° in anaerobic jar for 48

h.Identification was done according to Holt et al.[18] and MacFaddin [19].

The bacteria were grown in Skim Milk broth medium in a shaking incubator at 37C° for 20 hr . after which time the bacteria were washed twice with sterile PBS following centrifugation. Bacterial count were obtained using an improved Neubauer counting chamber. The culture was adjusted to 5×109 cell/ml and then stored at 4C° . [17] .

Expermintal animals:

White BALB/c rats , of either sex, (5-8)weeks old , were purchased from the Animal House of College of Science , University of Kufa. They were housed in groups of five under suitable conditions.

In vitro antagonism activity of L.acidophilus against C.albicans.

For antagonism activity digging agar method was used, 0.1 ml (containing approximately 1×10 8 cell/ml) of C. albicans was poured on sterile Petri dish , then thin layer of SDA was displaced in to these plates. The plates were incubated at 37C◦ for 24 h.

A cork borer was used to make pore (4×4 mm in diameter) on the surface of SDA. 0.1 ml of L. acidophilus (containing approximately 5×10 9 bacteria) was added to each pore . Plates were incubated at 37 C° 24 h. The inhibitory activity of L. acidophilus against C.albicans was determined by measuring the inhibition zones around each pore.[17,20]).

Oral feeding

Rats were fed 1×109 Lactobacilli in 0.2 ml PBS by gastric intubation using a feeding needle every day for 2 weeks. Control group of rats were fed with PBS . One day after the last feed , all rats were orally challenged with 1×108 C. albicans blastoconidia by topical application . Feeding was continued for an additional 6 and 14 days after challenge in BALB/c rat . At each time – point rat were killed to determine the level of colonization [17].

Oral infection

Rats were anaesthetized by chlorophorm, then they were inoculated orally with 108 live C. albicans yeasts in 20 µL of PBS. The infection was monitored for 21 days by swabbing the oral cavity with sterile cotton swabs moistened with sterile PBS olso plating on Sabouraud's agar plates . Agar plates were incubated for 48h at 37C◦ [21].

Assessment of oral infection

Rats were killed at various time-points to determine the number of C. albicans in the oral mucosa. The oral cavity (i.e. cheek, tongue and soft palate) was completely swabbed . After swabbing , the cotton end was cut off and then placed into an Eppendorf tube containing l ml of PBS. The yeast cells were resuspended before culture in serial 10 –fold dilutions on Sabouraud dextrose agar for 48 h at 37C◦ [17].

CFU were counted on Sabouraud's agar plates . The counts were assigned into five groups correlating with the level of recoverable yeasts from the oral cavity [21].

Statistical analysis

F test was used to analyze the quantitative data , P – values < 0.05 were considered significant .

Results

-In vitro antagonistic activity of L. acidophilus against C. albicans.

The antagonism effect of L. acidophilus against C. albicans was evaluated on SDA by digging agar method. Wide inhibitory zone was detected on SDA(3 cm in diameter) as in Fig -1. This indicate that L. acidophilus with high antagonism activity and was able to inhibit the growth of pathogenic C. albicans .

- Oral colonization of C. albicans.

White BALB /c rats were infected orally with 1×108 C. albicans yeasts and monitored for 21 days by swabbing the oral cavity. Results revealed that oral thrush were developed within the fourth day after infection as in Fig- 2. Swab culture showed higher number of yeasts recovered from the oral cavities of these lesions.

-Kinetics of oral infection by C. albicans in rats fed L. acidophilus .

The results in Fig-3 show that the level of colonization in the oral cavity in BALB/c rats fed L. acidophilus were similar to control (PBS-fed) rats 1 day after infection. A rapid decline in colonization level was detected at days 10 in the groups of rats fed L. acidophilus [compared with a 3- log higher number of yeasts in control rasts ($p<0.05$)]. By day 15, rats fed L. acidophilus had undetectable numbers of yeast in the oral cavity.

Fig (1): Inhibition growth of *Candida albicans* by *Lactobacillus acidophilus* on SDA.
A: *C. albicans* .
B: *L. acidophilus*.

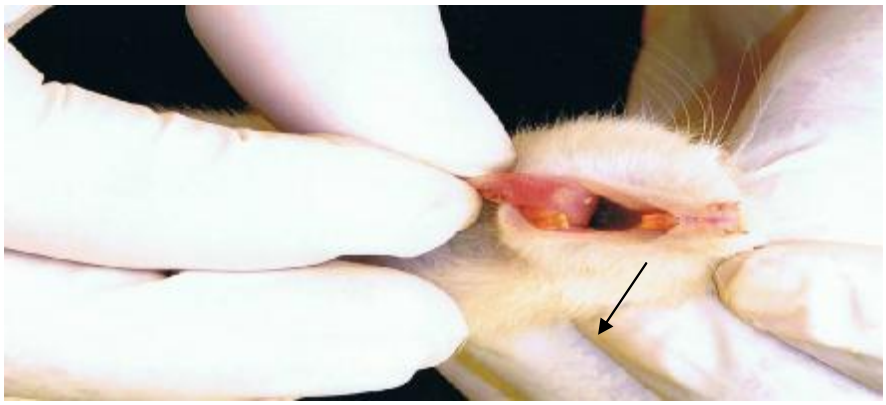
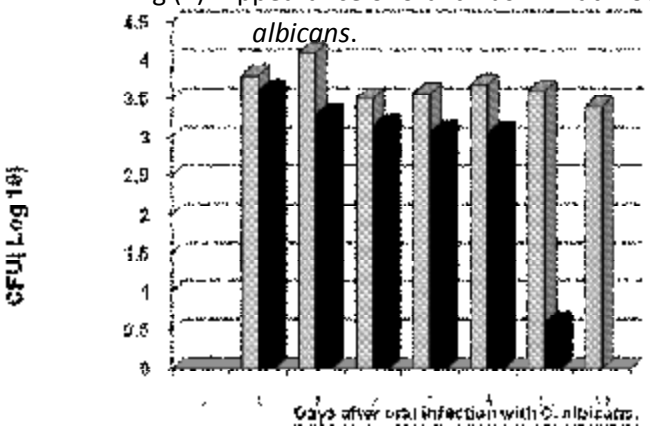


Fig (2): Appearance of oral thrush in rat inoculated with suspension of *Candida albicans*.



Fig(3):Elimination of *Candida albicans* **from oral cavities of BALB/c rats fed** *Lactobacillus acidophilus* **each day for 20 days after which time they were infected with 1×10** ^{8} *C .albicans***.**

Discussion

In the present study , the antagonism activity between L. acidophilus and pathogenic C. albicans was investigated using digging agar method. Results showed that L. acidophilus with high antagonism activity and inhibited the growth of C. albicans . Other workers noted the Inhibition of C. albicans by L. acidophilus in vitro [13] and also demonstrated in vivo [17].

Noverr and Huffnagle [4]. 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 .

Yamaguchi et al., [10] 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 (H2O2) production . [13].

Our study revealed higher level of oral colonization by C. albicans in BALB/c rats. This result in line with study of Farah et al., [21], However , they had reported higer level of oral candidiasis on the BALB/c and CBA/CaH athymic (nude)mice compared to euthymic control mice. Other studies of experimental C.

albicans infections of mucosa has shown small numbers of yeasts and hyphae colonizing the palate and tongue of normal (euthymic)mice. [22,23].

Thus the current study demonstrates that BALB/c rats are naturally susceptible to oral candidiasis and provide an excellent model to study various aspects of susceptibility and resistance to the disease.

It is generally believed that strains of lactobacilli participate in the control of the potentially pathogenic microorganism, including . C. albicans by colonizing alimentary tract and vaginal epithelium [8,9].Other investigators refer to the role of L. acidophilus in preventation of colonization and infection caused by pathogenic bacteria , Boris et al., [11] reported that vaginal lactobacilli inhibiting the growth of Escherichia coli and Streptococcus agalactiae in vitro . Oyetayo [24] also demonstrate that the use of faecal strains of L. acidophilus as alternative to the use of antibiotics since they can enhance the performance of rats and also prevent the incidence of diarrhoea associated with E. coli infection .

Our results revealed that feeding live L. acidophilus to BALB/c rats significantly shortens the duration of colonization of the oral cavity following inoculation with C. albicans when compared to control rats. Elahi et al., [17] noted that the accelerated clearance of C. albicans from the oral cavity of DBA/2 mice fed L. acidophilus correlated with an early appearance of mRNA for both interleukin (IL)-4 and interferon (IFN) - g , and their secreted products , from stimulated cervical node lymphocytes , and with the appearance of the effector molecules IFN-g and nitric oxide in saliva , as key parameters of effective immunity.

L.acidophilus induced increase in IFN-g and IL-12 secretion from the cervical nodes and higher early levels of salivary nitric oxide and IFN-g than did control mice or mice fed L. fermentum [17]. Gackowska et al. [6]

found that L.acidophilus was much stronger IFN-g inducer and with higher IL-12 stimulator than L.delbrueckii spp. bulgaricus and Bifidobacterium bifidum .

Elahi et al., [17]also noted that their is high level of nitric oxide prior to C. albicans challenge , following ingestion of L. acidophilus and this suggests that L. acidophilus stimulates additional mechanisms possibly involving macrophages , as enhanced secretion of IL-4 from stimulated T cells. Perdigon et al., [25] noted that Swiss mice feeding fermented milks with L.casei and L. acidophilus for 8 days (100µ g/day)showed an increase in both phagocytic and lymphocytic activity . All these support the notion that L. acidophilus have capacity to stimulate celluar and humoral parameters of mucosal protection , particulary in terms of salivary nitric oxide and IFN-g levels.

Data showed that the elimination of yeast from the oral cavity of rats was detected for at least 15 days after ceasing oral feeding with live L. acidophilus while study of Elahi et al. [17] reported that DBA/2 mice need at least 6 days for clearance of C. albicans from the oral cavity . The differences in results may be related to the difference in the strain of animals and strain of organisms used in the present study.

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