Original Article

Comparing the Protective Effect of the Conventional Pasteurized and Lactobacillus Acidophilus-fortified Pasteurized Yoghurts on *Candida Albicans*

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ABSTRACT

Statement of Problems: Candida species are the most common fungal pathogen in human's body. Therefore an accurate and immediate treatment seems to be necessary. Nowadays, alternative treatments, such as probiotics, are considered because of the adverse side effects of chemical medications. Probiotics are alive organisms which can be used for medical purposes and are added to different kinds of diary such as yoghurt. Lactobacill Acidophilus (LA) was detected form human's recourses many years ago and nowadays can be found in special kinds of milk, yoghurt, juice and food complementories.

Purpose: In this study we are to compare the effect of conventional pasteurized and lactobacillus acidophilus- fortified pasteurized yoghurt on *Candida Albicans* in vitro.

Materials and Method: *Candida Albicans* was isolated from 30 oral candidasis patients, cultured and prepared as standard suspension. Probiotic powder of LA in MRS Media after 24hrs led to 5×10^9 lactobacillus. LA-fortified yoghurt was prepared via adding lactobacills into 250 ml of pasteurized boiled milk after 8hrs in 37^{oc}.

We prepared 3 tubes and added standard suspension of Candida in each of them. Adding sterile yoghourt, conventional pasteurized yoghurt and LA-fortified yoghurt to the tubes respectively, we put them in 35^{oc} incubator for 48 hrs. We added the content of each tube in Sabouraud agar media and incubated in 25^{oc} for 72 hrs and then counted the colonies.

Results: There was a significant difference between the mean of colonies in each group during 5 days ($p = 0$, df = 4) and also on each day ($p = 0$, df = 2).

Conclusion: L.A. can inhibit colonization of Candida in vitro. In this study the most of Candida colonies were in the sterile yoghurt and the least of them were in LA fortified yoghurt.

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Introduction

Candida Albicans is the main cause of the majority of fungal infection in oral cavity. Oropharyngeal candidiasis is a common infection and currently ranks as the most common human fungal disease. Candida albicans, the major human pathogen of the genus candida, is a commensal yeast of the oral, gastrointestinal, and vaginal mucosa in healthy individuals and seems to be almost universally present [1]. But this microorganism may change from commensal to pathogenic microorganism in the mouth in relation to the oral and systemic conditions as being also shown by Kedir, et al [2]. The

high incidence of oropharyngeal candidiasis, highlights the need for the development of effective agents for its prevention.

There are several antifungal medications but the patients are mostly convenience using natural or herbal ones. Probiotics are defined as live microorganisms which when administered in adequate amount confer a health benefit on the host [3].

Several lactobacillus species are currently used as probiotics, i.e., live bacteria used as food supplements to provide beneficial effects to the host. Probiotic bacteria can produce bacteriocin-like compounds that inhibit infectious microorganisms [4]. They can adhere to epithelium of the alimentary tract and block adherence of pathogens [5] and they can stimulate host defense mechanisms [6-7].

Lactobacillus acidophilus (LA) is an important probiotic species, which has been reported to immune-deficient mice from orogastric candidiasis [7] and protect humans from *C.albicans* vaginitis [8]. Invitro treatment with yoghurt or exogenous lactoloacillus species (mainly LA) has been reported to alleviate the symptoms of vaginal candidosis [8-10] but few reports have investigated invitro effect of yoghurt on *C.albicans*.

According to the high prevalence of oral candidiasis specially in immune compromised patients and in convenience due to using local antifungal medications and the significant side effect of using systemic ones, introducing a kind of dairy product which has no side effect and adversely may have significant antifungal effect seems to be worthy enough to study on.

This study was designed to compare the invitro inhibitory effect of the conventional pasteurized and lactobacillus acidophilus-fortified pasteurized yoghurts on *C.albicans*.

Materials and Method

LA was obtained from the department of microbiology culture collection, Tabriz University of Medical Sciences, Iran. The bacteria were grown in Man-Rogosa-Sharpe (MRS) broth medium (Difco, Detroit, MI, USA) in a shaking incubator at 37°Cfor 20h after which time the bacteria were washed twice with sterile PBS following centrifugation. Bacterial counts were obtained using an improved Neubauer counting chamber. A total of five fields per sample were counted using a phase contrast microscope (Olympus, B×40, Japan) at 40× magnification. The culture was adjusted to 5×10^9/ml .and then stored at 4°C until use. The viability of bacteria was assessed by plating in serial dilutions aliquots of bacteria on MRS agar plates. Then 250 ml sterile cow milk (Pegah Company) was boiled at 90°C for 15 minutes and cooled to 37°C, aseptically. Probiotic yoghurt prepared by this 250 ml sterile cow milk included by 5×10^9 LA and incubated at 37°C for 8 hours.

The strain used in this study was *C. albicans*, isolated from samples taken from the palatal mucosa of a patient with denture stomatitis, examined at the Tabriz Dental University (Iran), using sterile paper points (DMS Dental Mirror Company, Ltd., Sligo, Ireland). The sample was transferred to vials containing 0.5 mL of reduced transport fluid (RTF) and sent to the Department of Microbiology, Tabriz University of Medical Sciences. The paper points were transferred to tripticase broth (Difco Laboratories, Detroit, Michigan) (5 mL) and incubated at 37°C for 24 hours. *Candida* strains were isolated on Sabouraud agar (Difco Laboratories). *C. albicans* were identified by a germ tube test and its ability to grow on cornmeal Tween 80 agar (terminal chlamydospores singly). The isolated strain of *C. albicans* was maintained in a 50%-glycerol liquid at −70°C. The content of these tubes was planted onto Sabouraud agar and incubated at 37°C for 24 hours. Each colony was inoculated in tripticase broth, which was then incubated overnight at 37°C.Then a suspension matching the turbidity of a 0.5 McFarland standard was prepared with phosphate buffered saline (PBS) [11].

From this suspension 2µl which correspond to 10^5 Colony Forming Units (CFU) was used as inoculum. Then three sterile laboratory tubes were inoculated with a final concentration of 10^5 CFU C. *albicans* diluted in 2µ PBS. We added 1ml sterile pasteurized yoghurt (Pegah Company) to the first tube, 1ml ordinary pasteurized yoghurt (Pegah Company) to the second and 1ml probiotic yoghurt to the 3rd tube and incubated them for 48h in 35°C.

Then the content of these tubes was planted onto sabouraud agar and incubated for another 72h in 25°C. The number of colonies was counted and expressed

Table 1 Levels of *C. albicans* colonization (Colony Forming Units) with different yoghurts during five days after incubation.

Type of Yoghurt \ Day	1	2	3	4	5
Sterile pasteurized yoghurt	1.55×10^5	1.64×10^5	1.9×10^5	2.11×10^5	2.26×10^5
Ordinary pasteurized yoghurt	1.47×10^5	1.63×10^5	1.83×10^5	2.01×10^5	2.19×10^5
Fortified yoghurt with LA	0.6×10^5	1.11×10^5	1.36×10^5	1.54×10^5	1.81×10^5

as colony forming units (CFUs) for 5 days after incubation in each tube and the total number of colonies in each day compared between three tubes. Viable counts were transformed to $\log_{10}$ CFU; the results, presented as the mean of $\log_{10}$ CFU, were compared by one-way ANOVA test. *p*-value <0.05 were considered significant.

Results

The level of *Candida albicans* colonization was increased up to the 5th day in each three tubes (Table 1) and none of these yoghurts could stop candida colonization. The highest level of candida colonization was seen with sterile yoghurt and the least number with probiotic yoghurt. The level of *C. albicans* colonization in the 3rd tube (probiotic yoghurt) was significantly lower than that of two other tubes (*p* <0.05) at days 1, 2, 3, 4 and 5 after incubation (figure 1) and according to this study L.A was able to inhibit colonization of *C.albicans.*

Discussion

The result of this study showed that probiotic yoghurt containing L.A significantly reduced the level of *C.albicans* colonization. This result was in accordance to Collins et al [12]. Their findings have shown that the growth of *C.albicans* was retarded at PH:7.7 by filtrates of L.A grown in casitone broth. They have suggested that L.A suppresses yeast in part by producing metabolites that inhibit *C.albicans* [12].

Elahi et al [13] have shown that feeding live L.A to an infection-prone mouse significantly shortens the duration of colonization of the oral cavity following inoculation with *C.albicans*. They have found that the accelerated clearance of *C.albicans* from the oral cavity of mice fed by L.A, correlated with an early appearance of mRNA for both IL4 and INF-γ and their secreted products from stimulated cervical node lymphocyte and with the appearance of the effector molecules INF-γ and NO in saliva [13].

Clancy and Cross showed that certain probiotic bacteria have a valuable capacity to drive the common mucosal system to enhance protection at distant mucosal sites [14-15]. This result is also in accordance to the data obtained in this research.

The variability in the capacity of different lactobacillus bacteria to stimulate cellular and humoral parameters of mucosal protection is noted, particularly in terms of salivary Nitric oxide (NO) and INF-γ levels. Previous studies in yeast infected murine model demonstrated a protective positive paracrine feedback loop between NO and IL4 production [16]. In the present study, none of utilized yoghurts could stop candida colonization to the relatively full extent.

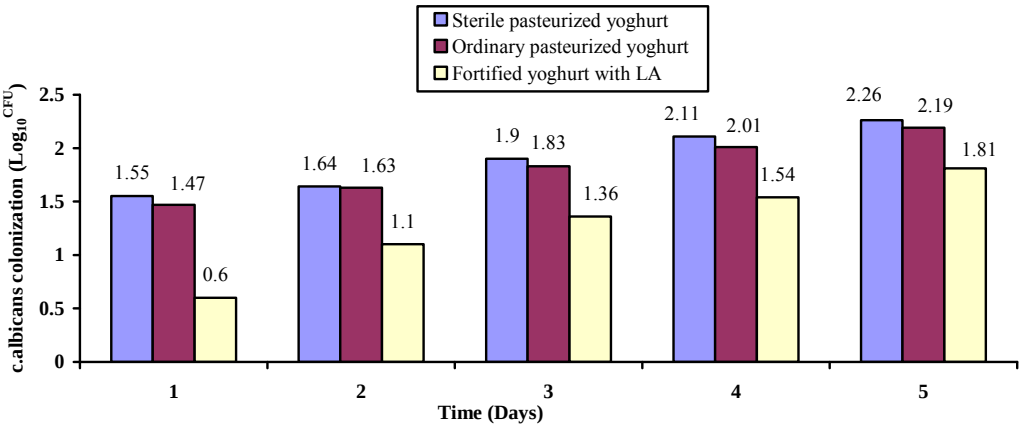

Figure 1 Effect of different types of yoghurt on *C. albicans* colonization (CFU) at days 1,2,3,4,5 post incubation.

Hamed et al [17] have suggested that yoghurt can significantly slow down the growth of *C.albicans* via introducing lactobacillus species into the reproductive tract milieu.

However, yoghurt does not completely eradicate the fungus. This is evidenced in mice with oestrogen-dependent vaginal candidosis which were treated by yoghurt through the experiment, but the infection remained persistent.

This finding is in accordance to the numerous previous studies, which have indicated that yoghurt treatment ameliorates the symptoms of vaginal candidosis [18-19].

Conclusion

L.A. can inhibit colonization of Candida in vitro. In this study the most of Candida colonies were in the sterile yoghurt and the least of them were in LA fortified yoghurt.

References

[1] Akpan A, Morgan R. Oral candidiasis. Postgrad Med J 2002; 78: 455-459.

[2] Kadir T, Uygun B, Akyüz S. Prevalence of Candida species in Turkish children: relationship between dietary intake and carriage. Arch Oral Biol 2005; 50:33-37.

[3] Reid G, Jass J, Sebulsky MT, McCormick JK. Potential uses of probiotics in clinical practice. Clin Microbiol Rev 2003; 16: 658-672.

[4] Tahara T, Oshimura M, Umezawa C, Kanatani K. Isolation, partial characterization, and mode of action of Acidocin J1132, a two-component bacteriocin produced by Lactobacillus acidophilus JCM 1132. Appl Environ Microbiol 1996; 62: 892-897.

[5] Bernet MF, Brassart D, Neeser JR, Servin AL. Lactobacillus acidophilus LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut 1994; 35: 483-489.

[6] Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 1996; 64: 5403-5405.

[7] Wagner RD, Pierson C, Warner T, Dohnalek M, Farmer J, Roberts L, et al. Biotherapeutic effects of probiotic bacteria on candidiasis in immunodeficient mice. Infect Immun 1997; 65: 4165-4172.

[8] Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing Lactobacillus acidophilus as prophylaxis for candidal vaginitis. Ann Intern Med 1992; 116: 353-357.

[9] Hughes VL, Hillier SL. Microbiologic characteristics of Lactobacillus products used for colonization of the vagina. Obstet Gynecol 1990; 75: 244-248.

[10] Williams AB, Yu C, Tashima K, Burgess J, Danvers K. Evaluation of two self-care treatments for prevention of vaginal candidiasis in women with HIV. J Assoc Nurses AIDS Care 2001; 12: 51-57.

[11] Catalán A, Pacheco JG, Martínez A, Mondaca MA. In vitro and in vivo activity of Melaleuca alternifolia mixed with tissue conditioner on Candida albicans. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105: 327-332.

[12] Collins EB, Hardt P. Inhibition of Candida albicans by Lactobacillus acidophilus. J Dairy Sci 1980; 63: 830-832.

[13] Elahi S, Pang G, Ashman R, Clancy R. Enhanced clearance of Candida albicans from the oral cavities of mice following oral administ-ration of Lactobacillus acidophilus. Clin Exp Immunol 2005; 141: 29-36.

[14] Clancy R. Immunobiotics and the probiotic evolution. FEMS Immunol Med Microbiol 2003; 38: 9-12.

[15] Cross ML. Immune-signalling by orally-delivered probiotic bacteria: effects on common mucosal immune-responses and protection at distal mucosal sites. Int J Immunopathol Pharmacol 2004; 17: 127-134.

[16] Elahi S, Pang G, Ashman RB, Clancy R. Nitric oxide-enhanced resistance to oral candidiasis. Immunology 2001; 104: 447-454.

[17] Hamad M, Muta'eb E, Abu-Shaqra Q, Fraij A, Abu-Elteen K, Yasin SR. Utility of the oestrogendependent vaginal candidosis murine model in evaluating the efficacy of various therapies against vaginal Cand-ida albicans infection. Mycoses 2006; 49: 104-108.

[18] Jeavons HS. Prevention and treatment of vulvovaginal candidiasis using exogenous Lactobacillus. J Obstet Gynecol Neonatal Nurs 2003; 32: 287-296.

[19] Hilton E, Rindos P, Isenberg HD. Lactobacillus GG vaginal suppositories and vaginitis. J Clin Microbiol 1995; 33: 1433