Antimicrobial Activity of Local Imported Yoghurts Against Candida Albicans

Abdulelah A. Al-Mayah , Eiman A. Saeed
Tamadir H. Wadi , Huda Sh. Farhan
University of Basra College of Pharmacy Department of Clinical Laboratory Sciences
abdulelah.suhain@gmail.com

Abstract

Twelve types of imported traditional yogurts and home-made yogurts are studied for antimicrobial activity against *Candida albicans*. The investigation showed that the presence of *lactobacillus spp*. in some types of used yoghurts, while lack in others. Antimicrobial activity of yoghurts with presence of *Lactobacillus* was established against *Candida albicans*. The metabolites produced by *Lactobacillus* were inhibited *Candida albicans* growth. Water solutions with different pH values (5, 7, 8) were used to cultivate *Candida albicans*, the results showed there is no effect of pH values on the growth of *Candida albicans*, while the presence of *Lactobacillus* and their secondary metabolites exhibited notable inhibitory effect against *Candida albicans* growth.

key wrds :**antimicrobials , yoyurts , yeast , lacto bacllus , candida**

الخلاصة

أستخدم أثنا عشر نوع من الألبان التجارية المستوردة من مصادر مختلفة وكذلك البان منزلية الصنع، لدراسة فعاليتها ضد المبيضات *Candida albicans*. أظهرت الدراسة أن الألبان المستوردة وكذلك اللبن منزلي الصنع يحتوي على العصيات اللبنية *Lactobacillus*، وخلت الأنواع الأخرى من الألبان من هذه العصيات.

أثبتت الدراسة الحالية وجود تأثير مثبط للنواتج الأيضية للعصيات اللبنية على نمو المبيضات، أذ تم أنماء المبيضات في محاليل مائية مختلفة الأس الهيدروجيني (٥، ٧،٨) ولوحظ عدم وجود أي تأثير تثبيطي لقيم الأس الهيدروجيني المختلفة على نمو المبيضات، في حين أظهر تواجد العصيات اللبنية ونواتجها الأيضية في الوسط تأثير تثبيطي واضح ضد المبيضات.

الكلمات المفتاحية : مضادات الميكروبات . المبيضات candida albicans

INTRODUCTION

Probiotic bacteria defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host like lactic acid bacteria (Meurman & Stamatova, 2007; Twetman & Stecksen-Blicks, 2008), such health promoting bacteria are added to different commercial dairy products such as milk, cheese and yoghurt. Possible action of probiotic bacteria in production of antimicrobial substances and activation and regulation of the immune response (Parvez *et al*., 2006; Allaker & Douglas, 2009). Bacterial antagonism may occur when growth of one bacterial species is hampered by components produced by another species. Lactic acid bacteria produce antimicrobial and some have the ability to produce hydrogen peroxide (H_2O_2) that can be toxic to organisms producing little or no H_2O_2 (Onwehand, 1998; Becker *et al.*, 2002; Meurman, 2005). Another important characteristic of *lactobacilluss* is production of proteinaceous bacterial substances which have intraspecies antagonistic effects. These substances have been known as bacteriocins (Vanderhoof & Rosemary, 2002; Reid, 2010).

Bacteriocin – like substances which normally have a broad spectrum of antimicrobial activity than bacteriocins can inhibit a wide range of Gram – positive, Gram – negative bacteria and *Candida albicans* (McGroarty, 1993; Birdsall, 1997; Vanderhoof & Rosemary, 2002).

The aim of the present study was to investigate the antibacterial effect of metabolites produced by *Lactobacillus* in yoghurt against growth of *C. albicans*.

MATERIALS AND METHODS

Samples of yoghurts

Twelve types of yoghurts used in the present study; 10 origins of commercial from local market in Basra city and 2 origins of home-made.

Yeast isolates

Candida albicans isolated from pulmonary infection patients, obtained from Research Laboratory in Clinical laboratory Sciences Department / College of Pharmacy.

First screening of *Lactobacillus*

Each type of yoghurt with amount of 0.01ml inoculated on MRS (deMan, Rogosa and Sharpe) agar medium with replications, samples then incubated under anaerobic conditions at 37◦C for (24-48)hr.

1. Ten ml from each used yoghurts dispensed in screw capped bottles, then inoculated 0.01ml of 10^6 cfu/ml (according to McFarland standard scale) from yeast suspension of *C. albicans*, then incubated at 37◦C for 48hr. Control samples performed by inoculating 0.01ml of *C. albicans* suspension onto SDA (Sabouraud Dextrose Agar), incubated at 37◦C for 48hr.
2. About 0.01ml from yoghurts inoculated with *Candida* suspension were cultured onto MRS agar and SDA plates, then incubated at 37◦C for 48hr (Harrigan & McCane, 1976).
3. Gram's staining and biochemical tests used to identify *Lactobacillus spp.* (Holt *et al*., 1994) and *Saccharomyces* yeast (Koneman *et al*., 1978).

Effect of pH on growth of *C. albicans* in yoghurts

1. The pH of all yoghurts was measured, and were found acidic (pH=5). From each type of yoghurts, 10 ml was dispensed in screw capped bottles, then inoculated with 0.01ml of 10^6cfu/ml from yeast suspension of *C. albicans* according to Mcfarland standard scale (Collee *et al*., 1996), then screw capped bottles incubated at 37ºC for 48hr.
2. From yoghurts that inoculated with yeast suspension of *C. albicans*, 0.01ml was inoculated onto the surface of MRS agar and SDA plates, then incubated at 37ºC for 48hr. Control plates performed by streaking 0.01ml from yeast suspension of *C. albicans* on SDA plates.
3. In another step, 10ml from each type of yoghurts suspended in screw capped bottles, then the pH of all used yoghurts was adjusted to pH 7 by adding drops of 10% NaOH, after that yoghurts inoculated with 0.01ml of 10^6 cfu/ml from yeast suspension of *C. albicans* and incubated at 37ºC for 48hr.
4. About 0.01ml taken from each type of yoghurts (that pH=7 and inoculated with yeast suspension) streaked onto MRS agar and SDA plates, incubated at 37ºC for 48hr. (Awan & Rahman, 2005).

pH effects on the growth of *C . albicans* in water solution

1. The pH adjusted to (acidic=5), (neutral=7), (alkaline=8) to three equal samples (10 ml) from sterilized distilled water that dispensed in screw capped bottles. Water samples then inoculated with 0.01 ml of 10^6 cfu/ml yeast suspension of *C. albicans*, all samples incubated at 37ºC for 48hr.
2. About 0.01ml from inoculated water samples with yeast suspension streaked on SDA plates then incubated at 37ºC for 48hr.

Lactobacillus spp. production of metabolites

1. MRS broth dispensed in screw capped bottles (10ml each) with replications in three equal groups, then inoculated with pure *Lactobacillus* isolates and incubated an aerobically at 37◦C in water bath-shaker for 7 days (Schillinger & Luke, 1989).
2. After incubation period, pH of three equal groups from the broth was adjusted to 5, 7, and 8 and inoculated with 0.01ml from yeast suspension, control samples performed by inoculating *C. albicans* suspension (without *Lactobacillus* metabolites) to broth samples, then incubated at 37◦C for 48hr.
3. About 0.01ml from broth samples that inoculated with *C. albicans* inoculated onto SDA, the results recorded after 48hr. at 37◦C.

RESULTS AND DISCUSSION

First screening of yoghurts

The results exhibited the presence of *Lactobacillus* as Gram positive long bacilli associated with *Saccharomyces* yeast in some types of yoghurts (Table 1), Fig.(1) and (2) while the other types lack the presence of two microorganisms and this may be related to pasteurization process.

Table (1). First screening of used yoghurts for bacterial and yeast presence

Types of yoghurts	The origin	Microbial contents
1. Kala	**Iran**	***Lactobacillus***
2. Avisa	**Iran**	***Lactobacillus***
3. Enas	**Turkey**	***Lactobacillus, Saccharomyces***
4. (Home-made)a	**Iraq**	***Lactobacillus, Saccharomyces***
5. (Home-made)b	**Iraq**	***Lactobacillus, Saccharomyces***
6. Activia	**Saudi Arabia**	**Gr+ rods, *Saccharomyces***
7. Al-Safi	**Saudi Arabia**	***Saccharomyces***
8. Mac	**Iran**	***Saccharomyces***
9. Manizan	**Iran**	***Saccharomyces***
10. Mast	**Iran**	**(-)**
11. Pacah Faris	**Iran**	**(-)**
12. Al-Wafrah	**Kuwait**	**(-)**

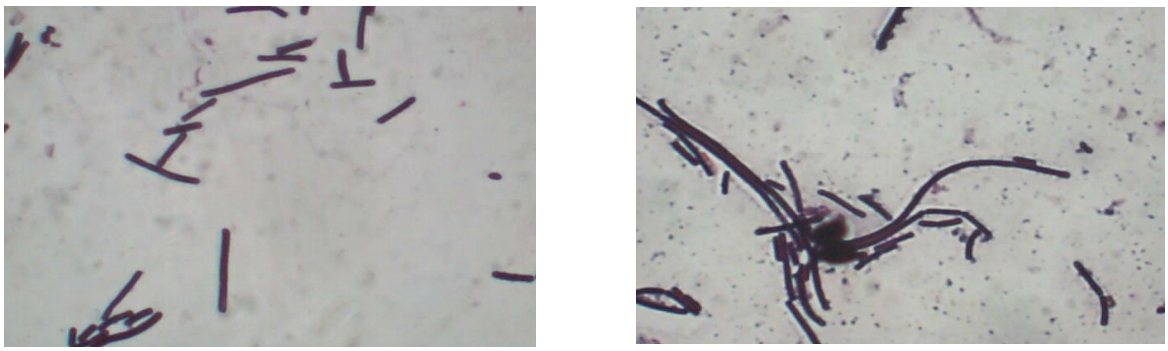

Fig. (1), (2): showed *Lactobacillus* isolates

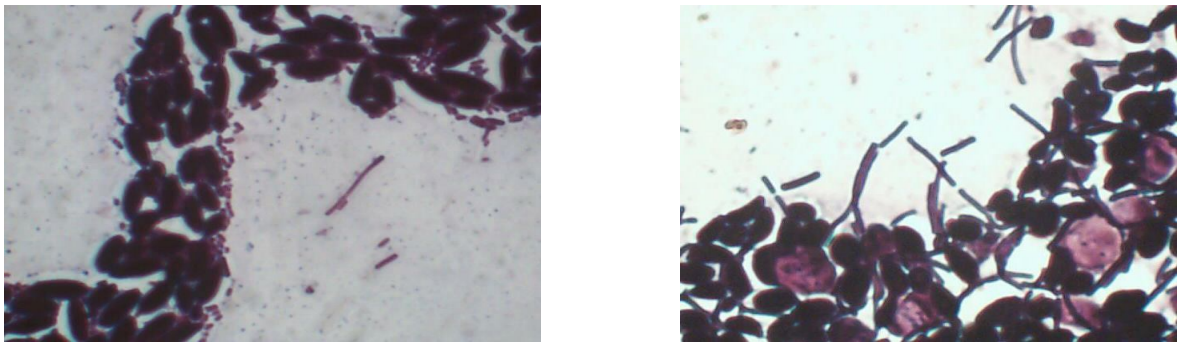

Fig. (3), (4): showed *Saccharomyces* yeast associated with *Lactobacillus* isolates

Biological activity of yoghurts against *C. albicans*

The results of inoculating yoghurts with *C. albicans* exhibited that yoghurts (type 1, 2, 3, 4, 5) contain *Lactobacillus* inhibit the growth of *C. albicans* and have the ability to eliminate it from the culture. While the other types of yoghurts (7, 8, 9, 10, 11, 12) that do not contain *Lactobacillus* have no effect on *C. albicans*, indicated by heavy growth of *C. albicans* on SDA culture medium. In type 6 of yoghurts, results showed the absence of growth of *Candida* in spite of it lack *Lactobacillus*, this may be related to the presence of *Bifidiobacterium* which have effective action on *C. albicans* growth Table (2). Yoghurts that contain non pathogenic bacteria like *L. acidophilus* help to fight yeast infection symptoms, have capability to stop them before it begin to growth, so yoghurts help to control *C. albicans***,** not allow to over populate and turn into a yeast infection (Hilton *et al.*, 1992; Shalev *et al*., 1996). Studies have shown that the daily ingestion of 150ml of yoghurt enriched with live *L. acidophilus* is associated with an increased colonization of friendly bacteria in the body. *Lactobacillus* have been used to treat or prevent infection of intestinal and uro-genital tract, also yoghurts often used by women with chronic vaginal *Candida* infection (Reid & Bruce, 2006).

pH effect on *C. albicans* in water solution

The results of cultivation of *C. albicans* suspended in distilled water (in pH 5, 7, 8) exhibited a good growth of *Candida* on SDA medium in acidic, neutral, and alkaline pH. This result indicate that the acidity of yoghurts do not have any affect on the growth of *C. albicans*.

Table (2). Biological activity of yoghurts against *C. albicans*

Yoghurts types	Yoghurts pH before adjustment	Microbial contents	
		MRS agar medium	SDA medium
1	5	*Lactobacillus*	No growth
2	5	*Lactobacillus*	No growth
3	5	*Lactobacillus, Saccharomyces*	*Saccharomyces*
4 (a)	5	*Lactobacillus, Saccharomyces*	*Saccharomyces*
5 (b)	5	Lactobacilli, *Saccharomyces*	*Saccharomyces*
6	5	Gv+ rods, *Saccharomyces*	*Saccharomyces*
7	5	*Saccharomyces*	*Saccharomyces, C. albicans*
8	5	*Saccharomyces*	*Saccharomyces, C. albicans*
9	5	*Saccharomyces*	*Saccharomyces, C. albicans*
10	5	No growth	*C. albicans*
11	5	No growth	*C. albicans*
12	5	No growth	*C. albicans*

Effect of *Lactobacillus* metabolites on the growth of *C. albicans*

The results revealed that secondary metabolites of *Lactobacillus* isolate have an inhibitory action against the growth of *C. albicans* in different pH values (5, 7, 8) in comparison with control samples (lack the presence of *Lactobacillus* metabolites) in which *C. albicans* showed heavy growth in acidic, neutral, and alkaline pH medium, this may be related to secondary metabolites that produced by *Lactobacillus* to the medium which inhibit the growth of *C. albicans* Table (3). These results compatible with other studies (Lowe & Arendt, 2004; Hatakka *et al.*, 2007; Slacanac *et al.*, 2010).

Table (3). Inhibitory effect of *Lactobacillus* metabolites on *C. albicans*

C. albicans	In presence of *Lactobacillus* metabolites			In absence of *Lactobacillus* metabolites		
	pH = 5	pH = 7	pH = 8	pH = 5	pH = 7	pH = 8
	No growth	No growth	No growth	Heavy growth	Heavy growth	Heavy growth

CONCLUSIONS

The present study showed :-

1. Lactobacilli isolated from yoghurts could produce metabolites have ability to inhibit growth of *C. albicans in vitro*.

2. Yoghurts acidity do not have a noticeable effect on *C. albicans* growth.

RECOMMENDATIONS

Further studies to be done to investigate the type of metabolites produced by *Lactobacillus* that found in yoghurts or other dairy products.

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