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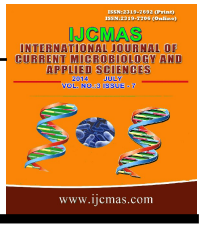

Original Research Article

The protective effect of probiotics (*Lactobacillus acidophilus* and *Saccharomyces boulardii*) against infections caused by *Staphylococcus aureus* In vitro and In vivo

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A B S T R A C T

Keywords

Probiotics
Lactobacillus acidophilus and Saccharomyces boulardii

Probiotics are microorganism that some have claimed provide health benefits when consumed. This study was aimed the protective effects of two types of probiotics (*Lactobacillus acidophilus and Saccharomyces boularidii*) by inhibitory material produced in vitro and in vivo against *Staphylococcus aureus* infections. 1-Invitro experiment: The in vitro experiment was performed on solid media by well diffusion method, plates was incubated 18 h at an incubation temperature 37c. 2-Invivo experiment: 8 mice were divided into three groups , the first included three animals, the second was three ,both were injected intraperitonially with 0.5 ml suspension of *lactobacillus acidophilus* and *Saccharomyces boulardii* respectively for five days, in addition to control group composed of two animals untreated. All groups were challenged with 0.2 ml of *Staphylococcus aureus* (10^8cell ml per ml).

Introduction

The World Health Organization's 2001 definition of probiotics is "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host (1). Probiotic microflora display numerous health benefits beyond providing basic nutritional value. They cooperatively maintain a delicate balance between the gastrointestinal tract and immune system. When this balance is disrupted, disease and inflammation result. Inflammation and over stimulation of the immune system by pathogenic bacteria are competitively inhibited by mucosal adherence of normal beneficial microflora (2). Many strains of probiotic microorganisms have been shown to inhibit growth and metabolic activity as well as the adhesion to intestinal cells of enteropathogenic bacteria (*Salmonella, Shigella, enterotoxigenic E. coli*, or *Vibrio cholerae*) to modulate (temporarily) the intestinal microflora and to have immunostimulatory or -regulatory properties(3). Probiotics may be commonly used as a therapeutic tool by health care practitioners in the not-too-distant future(2).

Lactic Acid Bacteria (LAB) are gram-positive bacteria with cell wall components such as peptidoglycan, polysaccharide, and teichoic acid, all of which have been shown to have immunostimulatory properties. In

addition to cell wall components, immunostimulatory effects were observed with antigens originated from the cytoplasms of some strains of LAB 9(4).

Saccharomyces boulardii is a tropical strain of yeast first isolated from lychee and mangosteen fruit in 1923 by French scientist Henri Boulard It is related to, but distinct from, *Saccharomyces cerevisiae* in several taxonomic, metabolic, and genetic properties(5). *S.boulardii* is sometimes used as a probiotic with the purpose of introducing beneficial active cultures into the large and small intestine, as well as conferring protection against pathogenic microorganisms in the host (4) However, in immunocompromised individuals, *S. Boulardii* has been associated with localized infection (6,7).

Materials and Methods

8 mice (Male 6 weeks-old Swiss blab\c) divided into 3 groups , the first two groups consisting of 3 mice ,the second 3 mice, in addition to control group consisting of 2 mice.

Probiotics

1-*Lactobacillus acidophilus*: (LBA) cultured on De man Rogsa sharp medium (MRS) at 37C for 48hrs.Washed with distilled water , killed at 100c for 30 min, and suspended in phosphate buffer saline (PBS) at desired concentration just before use(8).

2- *Sacchaomycets boulardii* :Maintained from Department of biology /College of science for women/University of Baghdad.

Pathogenic Bacteria

Staphylococcus aureus: Clinical isolates of *staph. Aureus* was isolated form infections and identified according to(9).

Susceptibility Testing: Susceptibility *of Staph. aureus* to 5 antibiotics including, Doxycyclne, Nitrofuran, Erythromycin, Cloxalexin, Cephalexin, were investigated by using Kirby-Bauer disk diffusion method and comparing their growth inhibition zones to those reported by CLSI (10).

In vitro experiment: Pathogenic bacteria was grown in the brain heart infusion broth, incubated overnight 37c and the concentration was diluted to 10^8 CFU /ml. Then spread on nutrient agar. One hundred microliter of 16 h supernatant (500 g for 10 min) LAB and *S.boulardii* separately were dropped into well on the nutrient agar and the plates were incubated overnight 37c ,the diameters of the inhibition zones were then measured(11).

Experimental infection

This study to test the protective effect of *L.acidophilus* and *Saccharomyces boularidii* against *Staph. aureus* in mice , 0.5 mg of suspension was injected in intraperitoneally (I.P.) 5 days before challenge with 0.2 ml of viable *Staph aureus* (10^8 cell/ ml)(12).

Determination of bacterial growth in the peritoneal cavity

The challenge of *Staph aureus* was to all groups of animals, 0.2 ml of viable pathogenic bacteria was injected I.P.24 h after the challenge, animals were sacrificed by Cervical dislocation . To follow bacterial growth in the peritoneal cavity , its contents were washed out with 5 ml of PBS, and the fluid was diluted 10- fold with PBS. Each dilution 0.1 ml was spread on nutrient agar plates (containing 0.4% glucose). The number of colonies in 5 ml of harvested fluid was expressed as Log 10 CFU.

Results and Discussion

The results of susceptibility to antibiotics showed that the isolate number 2 was more resistant than other three isolates. The diameters of inhibition zones were measured and compared with the zones of other isolates (Table 1). From these isolates, the isolate(2) were selected to test the protective effect of probiotics In vitro and In vivo.

Antibiotic resistance when bacteria change so antibiotics no longer work in people who need them to treat infections–is now a major threat to public health (13). Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, in particular as it pertains to pathogenic organisms.

Protection by probiotic bacteria and yeasts with immunostimulatory properties or the alleviation of symptoms and shortening of acute infections are perhaps the best-documented probiotic effects, and these have been demonstrated many times in the past in clinical studies fulfilling scientific requirements. Beneficial effects such as decreased frequency of infections and an increase in the production of rotavirus-specific antibodies have been demonstrated for a number of food (*Lactobacillus rhamnosus GG, L. casei Shirota, L. reuteri, L. acidophilus spec., Bifidobacterium animalis ssp. lactis BB-12,* and others) (14,15)

The results of the protective effect of *Lactobacillus acidophilus* and *Saccharomyces boulardii* against *Staphylococcus aureus* in solid media was studied. The results illustrated in table (2) showed the diameters of inhibition zones in which the inhibitory effect of the yeast on pathogenic bacteria was more that the effect of LAB (Fig1)(Fig1) .

The results of injection experiment showed that the survival of mice after the intraperitonial infection with *Staph.aureus* was augmented in the first and second group of mice that had been pretreated I.P with LAB and *Saccharomyces boulardii* separately five days previously. Mice became resistant to the infection with *Staph.aureus*.

The results of experimental infection showed that the growth of pathogenic bacteria was inhibited in the peritoneal cavity of animals pretreated with LAB and yeast. Whereas such inhibition of the bacterial growth was not observed in the control group.

These findings are in agreement with the previously reported result that showed that the administration of *Lactobacillus* or yogurt to young mice enhanced lung clearance of *P. aeruginosa* and phagocytic activity of alveolar macrophages (16). Some strains of LAB may affect pathogens by means of competitive inhibition (i.e., by competing for growth) and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer cells (17).Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory tract infections (18). Study suggested that probiotics, by introducing "good" bacteria into the gut, may help maintain immune system activity, which in turn helps the body react more quickly to new infections. Antibiotics seem to reduce immune system activity as a result of killing off the normal gut bacteria (19).

Table.1 Inhibition zone diameters of antibiotic sensitivity test for the 4 different isolates of *staph. aureus* measured in millimeter

Isolate no.	Do	F	E	Cx	Cl
1	21	22	24	0	0
2	12	13	0	0	0
3	26	23	22	0	0
4	24	18	20	0	0

Table.2 The inhibition zone diameters in the inhibitory effect of probiotics on *Staph. aureus* growth measured in millimeter.

Probiotics	inhibition zone diameters in millimeter	
Lactobacillus acidophilus	15	13
Saccaromyces boldarii	31	33

Fig.1 A/The effect of LAB on the growth of *Staphylococcus aureus*

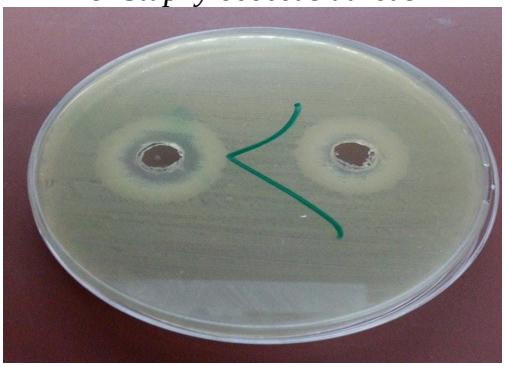

Fig.1 B/The effect of LAB on the growth of *Saccharomyces boulardii*

S. boulardii has been shown to be non-pathogenic, non-systemic (it remains in the gastrointestinal tract rather than spreading elsewhere in the body). It grows at the unusually high temperature of 37°C (20).A study showed that the *S. boulardii* has Anti-inflammatory effects , Interleukin 8 (IL-8) is a proinflammatory cytokine secreted during an E. coli infection in the gut. *S. boulardii* has been shown to decrease the secretion of IL-8 during an E. coli infection; *S. boulardii* could have a protective effect in inflammatory bowel disease. *Saccharomyces boulardii* may exhibit part of its anti-inflammatory potential through modulation of dendritic cell phenotype, function and migration by inhibition of their immune response to bacterial microbial surrogate antigens such as lipopolysaccharide (LPS)(21).

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