Antimicrobial effect of cell-free supernatant of *Lactobacillus* against isolated *Candida Spp.*

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ABSTRACT

Antimicrobial activity of the cell free culture supernatants (CFCS) was tested against the . *C.albicans* and *C. Parapsilosis* . the Zones of inhibition of probiotic (*Lactobacillus*) against EPEC were compared among 10^5,10^6,10^7 concentrations. Regarding *L. acidophilus*, the best zone of inhibition was obtained at 10^6 concentration. *L. plantarum* , the best zone of inhibition was obtained at 10^7 concentration. While the best inhibition zone of *L. rhamnosus* was obtained at 10^6 concentration and the Zones of inhibition of probiotic (*Lactobacillus*) against EAEC was compared among 10^5 , 10^6 and 10^7 concentrations. Regarding *L. acidophilus*, the best zone of inhibition was obtained at 10^7 concentration. In case of *L. plantarum*, the best zone of inhibition was obtained at 10^6 concentration. The best zone of inhibition was obtained at the concentration 10^6 *L. rhamnosus*. The Characterization of antimicrobial substances produced by Lactobacillus has been studded The result showed the bacteriocin only has inhibitory effect against pathogenic *E. coli* .

Keywords: Albicans and C. Parapsilosis ,Probiotic, Lactobacillus spp

Introduction

Probiotic refers to harmless live normal flora microorganism that provides a health benefit on the host, when administrated in adequate amounts and it leads to have nutritional advantage [1]. Probiotic microorganisms beneficially affect human health by improving the gastrointestinal tract (GIT) micro- biota balance and the defenses against pathogens. Additional health benefits attributed to probiotics are the stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities. There are strain most widely used as probiotics belong to genera *Lactobacillus*, *Bifidobacterium, Pediococcus, Lactococcus, Bacillus* yeast, and others [2]. Some of probiotic microorganisms are producing antimicrobial activity including organic acids e.g. lactic acid (LAs), and acetic acid (AAs), hydrogen peroxide (H_2O_2), bacteriocins, and others that inhibition of enteric pathogen [3].

A large number of studies have assessed the utility of probiotics in the prevention or treatment of certain clinical conditions; diarrheal illnesses are perhaps the best documented indication for probiotic therapy, particularly in the pediatric population. Approximately 50% to 80% of traveler's diarrhea cases are caused by bacteria, whereas theremaining cases are caused by viruses and protozoa. *E coli* are the most common cause of bacterial traveler's diarrhea. Clinical studies have shown inconsistent results in the use of probiotics for the treatment of traveler's diarrhea and the probiotics have preventive as well as curative effects on several types of diarrhea of different etiologies [4].

Materials and Methods

Lactobacillus spp was obtained from Microbiology Laboratory at College of Veterinary Medicine, Ferdowsi University by personel communication. It was used for Probiotic Bacteria.

Preparation of *Lactobacillus* cell free culture supernatants

1. Loopful bacterial culture was taken from agar plate, inoculated in MRSbroth and cells were grown to mid exponential phase for 24 h at 37°C under anaerobic condition.
2. The optical density of the standard cell suspension was adjusted with turbidity equals to McFarland standard no. 0.5.
3. To prepare the supernatant, 0.1 ml from this standard cell suspension was transferred to tube containing MRS broth then incubated for 24 hr., at 37°C., following of incubation.
4. The bacterial culture was subjected to centrifugation (10,000xg for 15 min, 4°C), filtered through sterilized 0.22 mm pore size membrane (Millipore)then plating on MRS agar showed no lactobacilli growth.
5. This freshly prepared cell free supernatant (stock solution) was used to check the inhibitory activity [6].

Antimicrobial activity of *Lactobacillus* isolates

Antimicrobial activity of *Lactobacillus* isolates were carried out according to the agar well diffusion assay as described previously [3]. Diarrheagenic *E. coli* were cultured in nutrient broth for 24 hours, and then cultured on nutrient agar by streaking technique.

1. Different concentrations of cell-free culture supernatant (CFCS) (100 µL) were placed into the wells of the nutrient agar and incubated at 37°C for 18 hours and the sterile MRS broth was used as negative control.
2. The diameter of the clear zones around each well was measured wider than 6 mm was considered as positive.

Characterization of antimicrobial substances producedby *Lactobacillus*.

The supernatant was aliquoted into five tubes:

A-First tube was treated with 1 mg /mL trypsin to determine the bacteriocin production.

B- Second tube was adjusted to pH 6.5 ± 0.1 with NaOH.

C-Third tube was treated with 0.5 mg /mL catalase for 30 min at 25 °C to determine hydrogen peroxide production.

D- Fourth tube was adjusted to pH 6.5 ± 0.1, treated with catalase andtrypsin.

E- Fifth tube was used as positive control (non-treated). Antimicrobial activity was carried out according to the agar well diffusion assay[7].

Statistical Analysis

The data of the present study was analyzed statistically by statistic package for social science (SPSS) version 27 program using chi-square test (X^2) and two-way ANOVA & Least significant differences (LSD). The level of significance was set to 5%. $P<0.05$ was considered significant while $P>0.05$ was considered as non-significant [7].

Results and Discussion

Evaluation of Antibacterial Activity by Probiotic (Lactobacillus) Antimicrobial activity of the cell free culture supernatants (CFCS) was tested against the *C. albicans* and *C. Parapsilosis* .The cell- free neutralized supernatant (CFS) of Lactobacilli (10^5,10^6,10^7) were inhibited the growth all *yeast* isolates by well diffusion method. It was also noticed that, L. acidophilus showed the strongest Antimicrobial activities against pathogenic *C. albicans* with different degrees of inhibition zones in comparsion with each of *L. rhamnosus* and *L. Plantarum* , while, *L. rhamnosus* revealed strongest Antimicrobial activity against pathogenic *C. Parapsilosi* table (1-1 and 1-2).

Table (1-1): Number of inhibited isolates of *C. albicans* (N=27) with cell free supernatant (CFS) of probiotic *Lactobacillus spp*

Lactobacillus spp	Number isolates of inhibition and %			X^2	P value
	10^5	10^6	10^7		
L. acidophilus	13(48.14)	19(70.37)	7(25.92)	4.82	0.09
L. plantarum	0(0)	15(55.55)	4(14.81)	13.37	0.001*
L. rhamnosus	0(0)	15(55.55)	5(18.51)	9.51	0.009*
X^2	16.57	1.26	0.833		
P value	0*	0.53	0.659		

10^5,10^6,10^7= cell free supernatant concentration
* Significantly difference at P<0.05

Table (1-2): Number of inhibited isolates of *C. Parapsilosis* (N=13) with cell freesupernatant (CFS) of probiotic Lactobacillus spp

Lactobacillus spp	Number isolates of inhibition and%			X^2	P value
	10^5	10^6	10^7		
L. acidophilus	0(0)	12(92.30)	3(23.07)	28.06	0*
L. plantarum	1(7.67)	6(46.15)	0(0)	10.62	0.005*
L. rhamnousus	2(15.38)	11(84.61)	5(38.46)	12.25	0.002*
X^2	2.15	9.1	5.86		
P value	0.341	0.011*	0.053		

10^5,10^6,10^7= cell free supernatant concentration
* Significantly difference at P<0.05

Zones of inhibition of probiotic (Lactobacillus) against *C. albicans* were compared among 10^5,10^6,10^7 concentrations. Regarding *L. acidophilus*, the best zone of inhibition was obtained at 10^6 concentration. *L. plantarum* , the best zone of inhibition was obtained at 10^7 concentration. While the best inhibition zone of *L. rhamnosus* was obtained at 10^6 concentration, as shown in table (1-3).

Table (1-3): Zones of inhibition for *C. albicans* (N= 27) with a cell freesupernatant of probiotic *Lactobacillus spp.*

Lactobacillus spp	**Zones of inhibition for C. albicans**		
	10^5	**10^6**	**10^7**
L. acidophilus	19.92±0.72Aa	2.7Ab.±30.55	25.92±1.97Ac
L. plantarum	0±0Ba	16.9±0.90Bb	34.3±1.88Bc

L. rhamnosus	0±0Ba	36±1.56Cb	24.15±1.51Ac
LSD(P<0.05)			2.58

10^5,10^6,10^7= Diameter of inhibition zone (mm) .Means with different capital letters n the same column and small letters inThe same row are significantly different.

Zones of inhibition of probiotic (*Lactobacillus*) against *C. Parapsilosis* was compared among 10^5 , 10^6 and 10^7 concentrations. Regarding *L. acidophilus*, the best zone of inhibition was obtained at *plantarum*, the best zone of inhibition was obtained at 10^6 10^7 concentration. In case of *L.* concentration. The best zone of inhibition was obtained at the concentration 10^6 *L. rhamnosus*, as shown in table (1- 4).

Table (1-4): Zones of inhibition for *C. Parapsilosis* (N= 13) with a cell free supernatant of probiotic Lactobacillus spp

Lactobacillus spp	**Zones of inhibition for *C. Parapsilosis***		
	10^5	**10^6**	**10^7**
L. acidophilus	0±0Aa	28.28±0.75Ab	42±1Ac
L. plantarum	19±1Ba	38.57±0.92Bb	0±0Bc
L. rhamnousus	15±0.57Ca	23.5±1.3Cb	20±0.31Cc
LSD(P<0.05)			1.38

10^5,10^6,10^7= Diameter of inhibition zone (mm)

Means with different capital letters in the same column and small letters in thesame row are significantly different

This result is agree with 13 Davoodabadi *et al*. (2020) who demonstrated that the Lactobacillus characterized by inhibitory activity against the Candida albicans isolated from oral candidiasis .These strains could be used as probiotic to help in preventing the oral infections caused by Candida. A similar results from study by **(** Jain *et al* .,2017), included isolates of *L. casei,L. delbrueckii, L. fermentum, L. plantarum, and L. pentosus,* that used the antibacterial activities of cell-free supernatants (CFSs) test for all the pathogenic isolates. The test was performed through standard agar-well diffusion assay, against human. Other study suggested that none of lactobacilli cell-free supernatant (CFS) has an inhibitory activity against four Candida spp., these species are *C C.tropicalsC. Parapsilosis .albicans C.kruse* (14).The result disagree with (15) that demonstrated *L. acidophilus* inhibited *Streptococcus agalactiae* and *P. aeruginosa* and no antimicrobial effect on against the *Candida albicans*.Antimicrobial activity is one of the most important selection criteria of probiotics. Antimicrobial effects of Lactic acid bacteria are incurred by producing some substances such as organic acid (lactic acetic, propionic acids, carbon dioxid, hydrogen dioxide, diaacetyl, low molecular weight antimicrobial substances and bacteriocin). Probiotics including Lactobacillus, Bifidobacterium and Streptococcus spp are known to be inhibitory to the growth of a wide range of intestinal pathogens in human. In addition to the favorable effects against diseases caused by an imbalance of the gut microflora(16) Other strategies used by Lactobacilli are the iron inhibitory effect for *Candida* and other pathogens by the regulation of intracellular iron concentration (17) . This effect take place due to the ironmetabolic need in Lactobacilli, the iron plays an important role in the pyrimidine and purine metabolism, therefore *lactobacillus* utilize it during the

growth in medium containing concentration of iron.

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