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COMPARATIVE STUDY BETWEEN THE EFFECT OF THE LACTIC ACID BACTERIA AND NON AGAINST SOME PATHOGENIC BACTERIA

Enas Ibrahim Jasim, Duaa Suhail Shawket,Yusra M.B. Mohsin, Basam Basim Mohammed, Dhilal Abdalelah Ibraheem, Mustafa Anwer Hussein
Department of Biology/College of Science/Al-Mustansiriyah University/ Baghdad- Iraq.
Corresponding author email: Enas_ht@yahoo.com

ABSTRACT
Two lactic acid bacteria (LAB) isolates (*Lactobacillus* & *Enterococcus Faecalis*) were isolated from different sources (fermented cucumber, infant stool and one *Enterococcus Faecalis* from environmental source). All isolates were tested for their cell free supernatant (CFS) activity against some pathogenic bacteria by Agar well diffusion assay. Additionally, CFSs were concentrated 3 times and labeled as CFS 1, CFS 2 & CFS 3 in addition to CFS to detect their activity. The results showed that the CFSs of *Lactobacillus* showed the higher antibacterial activity, as compared with the CFSs of *Enterococcus* (from infant stool) but more than environmental *Enterococcus* which had no activity on all the test bacteria except against *staphylococcus aureus*, so All CFS of LAB displayed numerous antibacterial activity more than non LAB like environmental *Enterococcus*.

KEYWORDS: Environmental *Enterococcus*, Cell Free Supernatant, *Lactobacillus*, Pathogenic bacteria, Infant stool.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of gram positive bacteria including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and *Streptococcus*. The general description of bacteria included in the group is gram–positive, no spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Recent taxonomic revisions of these genera suggest that lactic acid bacteria comprise the genus *Enterococcus* [1]. Consumption of food containing live bacteria is the oldest and still most widely used way to increase the number of advantageous bacteria called "probiotics". Noteworthy, there are large numbers of probiotic foods that date back to ancient times, the quest to find food ingredients with valuable bioactive properties has created interest in lactic acid bacteria with probiotic attributes such as antimicrobial activity against pathogenic microorganisms, LAB are the most prominent non-pathogenic bacteria that play a vital role in our everyday life, from fermentation, preservation and production of wholesome foods and vitamins to prevention of certain disease and cancer due to their antimicrobial action[2]. Probiotic are live microorganisms which have been found to confer a health benefit on the host when administered in adequate amounts, probiotics are mainly used to reinforce or re-establish the gut microbial balance especially when the hosts are confronted with challenges or stress[3]. Several researches have been conducted for investigating the anti–pathogenic activity of *Lactobacillus* spp. as probiotic bacteria both *in vitro* and *in vivo*[4]. *Enterococcus* is a lactic acid bacterium that is a normal inhabitant in the gut and that shows effects against enteropathogens, in the same time enterococci are a major colonizer of animal and human intestinal tracts[5]. In an earlier study, we found that an LAB complex (*Enterococcus faecium* 6H2, *Lactobacillus acidophilus* C3 and *Lactobacillus fermentum* NC1) alone or combined together had probiotic properties on pathogenic bacteria in animals[6]. Although, there is a lot of research about antimicrobial activity of LAB, against UTI pathogens[7] and against bacterial contamination of cosmetic tools [8] but few researches were dedicated towards the use of more than one genus from different sources of isolation as antimicrobial agents against pathogenic bacteria. Therefore, the aim of this study was to investigate the antibacterial activity of bacteria isolated from (fermented cucumber, infant stool, environmental) samples towards some pathogenic bacteria.

MATERIALS & METHODS

Isolation and Collection of LAB

Lactobacillus: it was isolated from fermented cucumber. Middle size pieces of cucumber were cut by sterile knife, and then pieces were fermented in a sterile container containing distilled water with 3% of Na Cl under 37°C in the incubator for 3 days. After that, MRS broth tubes were inoculated with 1% of fermented cucumber, incubated in anaerobic conditions at 37°C for 48 hrs. The isolate was diagnosed depending on Darsanaki[9].

***Enterococcus faecalis*:** two isolates of this bacterium are involved in the study. These isolates were obtained from postgraduate studies laboratories in the Department of Biology/Al-Mustansiriyah University. The first isolate was normal flora isolated from infant stool, while the second one was isolated from an environmental source. The diagnosis of these isolates was confirmed according to Panda [10].

The three isolates of lactic acid bacteria were called: Lb (*Lactobacillus*), EE (Environmental *Enterococcus*), and NFE (Normal flora *Enterococcus*).

Preparation of Cell Free Supernatant (CFS)

MRS broth tubes were inoculated with (Lb, EE, NFE), then incubated in anaerobic conditions at 37°C for 48 hrs., these tubes were centrifuged at 4000 rpm for 20 mins, Cell Free Supernatant (CFS) was collected and the pellet was discarded.

Cell Free Supernatant Concentration

Supernatants of CF were concentrated into once, twice and triple concentrations by incubated in an oven at 40°C. These different concentrations were used to measure the antibacterial activity of LAB isolates against pathogenic bacteria. CFS was labeled with CFS, CFS1, CFS2, CFS3 (non concentrated, once, twice, triple concentrations; respectively).

Tested Pathogenic Bacteria

Pseudomonas auruginosa, E. coli, Staphylococcus aureus were obtained from Postgraduate studies laboratories in the Department of Biology /Al-Mustansiriyah University and tested under antibacterial activity of CFS of LAB isolates by agar well diffusion assay.

Determination of Antibacterial Activity of Lb–EE–NFE against Test Bacteria

Agar well diffusion assay was made to determine the antibacterial activity of CFS against test bacteria. Depending on this method, Muller Hinton agar plates were streaked by 0.1 ml of bacterial inoculums for each plate. Wells were made by using a sterile cork borer and filled with CFS, CFS1, CFS2, and CFS3. The plates were incubated at 37°C for 18- 24 hrs., and the results were read by measuring the diameters of inhibition zones around the wells.

RESULTS & DISCUSSION

According to the recent study, all the test bacteria did not respond to CFS of EE. Also, CFS1 and CFS2 do not reveal any activity while CFS3 was active against *Staphylococcus aureus* only (Table 1, Figure 1). This result means that EE has weak activity. On the contrast, two other studies confirmed high activity of enterococci [12]. Those studies also reported high probiotic levels of activity whereas we reported low activity level of CFS3. These results did not suggest similarities around the globe.

TABLE 1: Antibacterial activity of environmental *Enterococcus fecalis* (EE) against test bacteria

Test bacteria	*Enterococcus fecalis* (EE) Inhibition zone (mm)			
	CFS	CFS1	CFS2	CFS3
Pseudomonas aeruginosa	-	-	-	-
Staphylococcus aureus	-	-	-	20
E.coli	-	-	-	-

(-) = No growth; CFS, CFS1, 2, 3 = not concentrated cell free supernatant, once, twice, triple concentrated

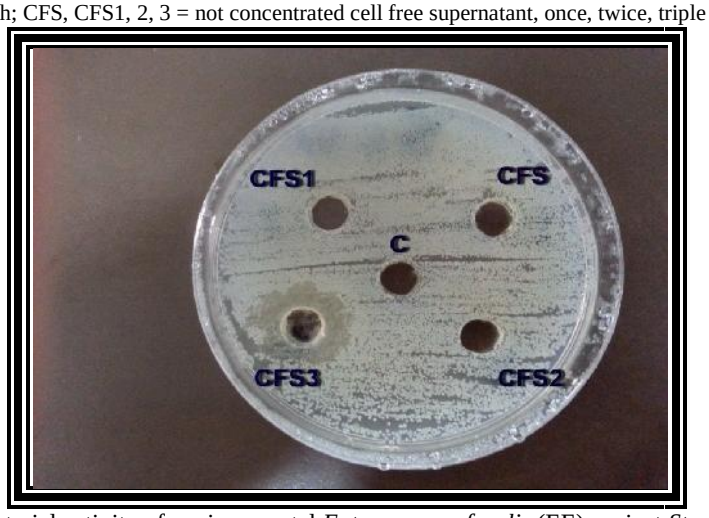

FIGURE 1: Antibacterial activity of environmental *Enterococcus fecalis* (EE) against *Staphylococcus aureus*
CFS, CFS 1, 2, 3 = not concentrated cell free supernatant, once, twice, triple concentrated; C = control

Interestingly, the result indicated that the test Gram+ve bacteria (*Staphylococcus aureus*) are highly sensitive in compared to Gram–ve (*Pseudomonas aeruginosa, E. coli*). The absence of inhibitory activity against Gram negative bacteria is not surprising as most of LAB substances inhibit the growth of closely related Gram positive bacteria.

Authors supposed that this may be due to composition of the cell wall among Gram positive and negative, the second have an outer phospholipids membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial substances. On the other hand, the Gram positive bacteria are more susceptible having only an outer peptidoglycan layer which is not effective permeability barrier, therefore, the cell wall of gram negative organisms are more complex in lay out that the gram positive ones, acting as diffusion barrier and making them less susceptible to the antimicrobial agent than of gram positive [10].

As the result indicated in table (2), the diameters of the inhibition zones were varied, they ranged between (12-17) mm, and this revealed that the normal flora *Enterococcus feacalis* inhibited all the tested pathogenic bacteria according to Jatkauskas and Vortniakien[13]. Similar study

was carried out which studied the activity of LAB on some bacteria like *Pseudomonas, Staphylococcus and E.coli* [14].

TABLE 2: Antibacterial activity of normal flora *Enterococcus fecalis* (NFE) against test bacteria

Test bacteria	*Enterococcus fecalis* (NFE) Inhibition zone (mm)			
	CFS	CFS1	CFS2	CFS3
Pseudomonas aeruginosa	13	14	14	14
Staphylococcus aureus	12	13	14	17
E. coli	11	12	12	15

CFS, CFS 1, 2, 3 = not concentrated cell free supernatant, once, twice, triple concentrated

Although CFSs of *Lactobacillus* displayed antibacterial activity against all test bacteria by the agar well diffusion assay, they showed high levels of activity against test bacteria when they were concentrated three folds (CFS3). The antibacterial activity remained active in CFS and increased by increasing the concentration of CFS (Table 3, Figure 2).

TABLE 3: Antimicrobial activity of *Lacobacillus* (Lb) against test bacteria

Test bacteria	*Lactobacillus* (Lb) Inhibition zone (mm)			
	CFS	CFS1	CFS2	CFS3
Pseudomonas aeruginosa	15	16	17	20
Staphylococcus aureus	15	20	20	22
E. coli	14	16	18	20

CFS, CSF 1, 2, 3 = not concentrated cell free supernatant, once, twice, triple concentrated

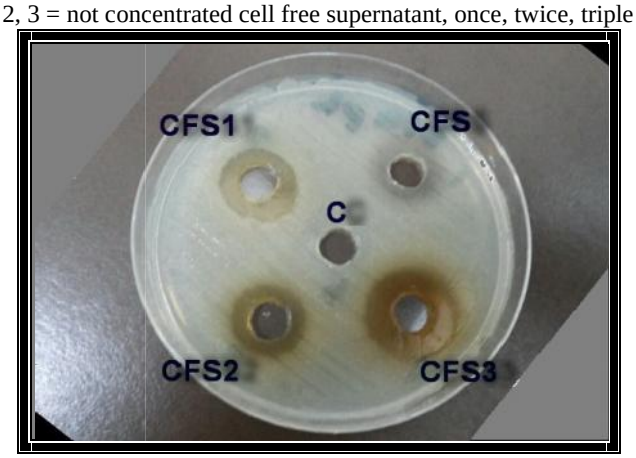

FIGURE 3: Antibacterial activity of *Lactobacillus* (Lb) against *Staphylococcus aureus*
CFS, CFS1, 2, 3 = not concentrated cell free supernatant, once, twice, triple concentrated; C = control

The action of LAB generally and *Lactobacillus* especially is attributed to the combined action of a range of antimicrobial metabolites, these include many organic acids such as lactic, acetic, and propionic acids produced as end products which provide an acidic environment unfavorable for the growth of many pathogenic bacteria [15]. Acids are generally thought to exert their antimicrobial effect by interfering with the maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic functions, they have a very broad mode of action and inhibit Gram- positive and negative bacteria as well as yeast and molds[16]. In addition to that and in particular, H2O2 can have a strong oxidizing effect on membrane lipids and cellular proteins and is produced using such enzymes as the flavor protein oxidoreductases NADH peroxidase, NADH oxidase and α- glycerophosphate oxidase. Obviously, each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens to overcome before they can survive and / or proliferate or beverage. Since any microorganisms may produce a number of inhibitory substances, its antimicrobial potential is defined by the collective action of its metabolic products on undesirable bacteria [17-19]. All these factors enable *Lactobacillus* to use as probiotic against pathogen because some resources define the probiotic in general terms as a group of requirements have been identified as important properties for lactobacilli to be effective probiotic organisms, these include the ability to: adhere to cells, exclude or reduce pathogenic adherence, persist and multiply , produce acid , resist pathogenic microorganisms, be safe and therefore noninvasive, noncarcinogenic and nonpathogenic and, co aggregate and form a normal [20-24].

CONCLUSION

- Not concentrated and concentrated CFSs of EE had no activity on all the test bacteria except *Staphylococcus aureus* which was sensitive to CFS3 only.

- All CFSs of the normal flora *Enterococcus feacalis* inhibited all the tested pathogenic, the diameters of the inhibition zones were varied.
- All CFSs of *Lactobacillus* displayed numerous antibacterial activities against all test bacteria. The antibacterial activity increased by increasing the concentration of CFS.
- This study confirmed the presence of probiotic action of lactobacilli isolates.
- Gram + ve bacteria (*Staphylococcus aureus*) are highly sensitive in compared to Gram–ve (*Pseudomonas aeruginosa, E. coli*).

RECOMMENDATIONS

- Probiotic producing cultures of LAB isolates must be characterized and purified for getting the active substances responsible for the activity.
- Probiotics can represent an effective and safe alternative to the use of synthetic substances like: antibiotic.

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