Determination of Probiotic Potential and Anticancer Activity of *Lactobacillus* Isolated from Cheese and Yogurt

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Abstract

In this study, microorganism was isolated from cheese and yogurt, cultured on specific culture media, then recognized molecularly through 16S rDNA f quality sequencing. The supernatant of *Lactobacillus* strains contained probiotic activity such as anticancer effects against cancer cell lines such as HeLa, RD and normal cell line (REF)cells and antibacterial. The cytotoxicity effects were assessed by MTT stain and apoptosis in mitochondria. The supernatant showed had anticancer activity against the tested cell lines ($P< 0.05$) with no significant cytotoxic effects against normal cell line .

Key words*: lactobacillus, cheese, yogurt, anticancer, apoptosis*

Introduction

Probiotic microorganisms consider fermented food products and are known to offer functional effects on mucosal damages, specifically preventing the effects of cancer on the digestive tract [1,2]. Probiotic microbes, which are known to lead to the generation of therapeutic anti-carcinogenic compounds, are primarily dependent on determination the relationship between probiotics and colorectal cancer [3,4].The present examination was planned to survey the fprobiotic and fanticancer exercises of *Lactobacillus*f.

Material and Methods

Isolation of *Lactobacillus*

42 isolates **of *Lactobacillus*** were gatheredf from dairy items which were gathered from various markets in Baghdad incorporate new drain of bovinesf, imported yogurtf (fActiviaf), localf yogurtf (fcanonf) and cheese f. For all isolates, about 1 gm or 1 ml was taken from source and was grown in 9 ml MRS broth (Himedia-India), and subcultures were done to get pure isolates by streaking petri dishs. The culture was incubated anaerobically at 37 °C for 24- 48 hrs. Depending on the cultural, microscopical examinations and biochemical tests, 42 strains were identified as *Lactobacillus* spp. [5].

preparation of Cell- Free culture supernatant (CFCS):

The isolates were inoculated in MRS broth for 24 hrs. at 37ºC . Bacterial cells were evacuated by centrifuging the culture at 8000 rpm for 15 min at 4ºC. pH of fsupernatant was adjusted at 6.5-7.0 by the faddition of 1N NaOHf and supernatants were filtrated through milliporef 0.22 µmf [6,7].

DNA extraction and Identification by PCR

DNA was extracted from *Lactobacillus* strains. DNA mini kit Geneaid, (Korea) was used according to the manufacturer's instructions [20]. PCRf intensificationf conditions were fperformed by [8,9].

Antibiotic Tests

The antibiotic susceptibility of the strains was examined by disc diffusion method against some antibiotics such as: Amoxicillin Clavulanic acid, Erythromycin, Imipenem, Ceftazidime, Vancomycin, Clindamycin, Chloramphenicol and Cefotaxime. Inhibition zones were compared with Standards of Antimicrobial Disk Susceptibility tests [10,11].

Probiotic antimicrobial assay

The diffusion method was used to determine inhibition effects for bacterial extraction toward many pathogenic

bacteria such as *Escherichia coli, Streptococcus spp, Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Candida albicans* isolates which were obtained from lab of graduate studies in Biology Department /Sciences Collage / Baghdad University/ Iraq. Culture media were used to inoculate pathogenic bacteria and fungi. Wells were made in the media to inoculate 50 µL of CFCS and incubated at 37°C for 18 hrs.. After that measurement of inhibition zones was performed [12].

Cytotoxicity by MTT assay

Cytotoxic impact of the **CFCS** on proliferation of the adherent cells in 96-well microtiter plate had been performed by [13,14].

Apoptotic cell detection

The dye of Mitocapture reagent kit would be concentrated in the mitochondria. The assay was carried out according to [15].

Statistical Analysis

One route of analysis of ANOVA was performed to test whether group variance was significant or not. Statistical analysis was performed by utilizing the program SPSS. Differences were considered significant at $P< 0.05$. **[16]**

Results and Discussion

Isolation and identification

Results of current study demonstrated that 42 isolates which were isolated from cheese and yogurt confined Gram-positive, bacilli. Catalase, gelatinase, and indole tests were negative, whereas, both Arginine and Skim milk coagulation tests were positive (table1). Molecular identification was depended on 16S rDNA sequencing, belong to *lactobacillus spp*. figure (1)

Antibiotic susceptibility

This examination revealed that this bacteria which was isolated from cheese and yogurt, was sensitive to vancomycin, erythromycin, amoxicillin clavulanic acid, clindamycin, and Imipenem. However, it was resistant to chloramphenicol and with intermediate susceptibility to cefotaxime, ceftazidine (table 2) . Here, it was affirmed that tested isolate can be considered as probiotics, possesses strong antibacterial and anticancer properties.[17].

Probiotic antimicrobial activity

Antimicrobial activity of CFCS of the *lactobacillus spp* isolates was tested against some pathogenic microorganisms, and showed the capacity to have abroad range of inhibition activities against isolates *E coli, Streptococcus spp, S aureus, P aeruginosa*, and *Candida albicans* (table 3). CFCS demonstrated inhibition zones which were greater than 18 ,13,10 mm against *E. coli*, *S. aureus* and *Streptococcus spp.* respectively, but did not affect *candida albicans* and *P. aeroginosa*. Lactobacilli can inhibit pathogen colonization and consequently prevent contamination. Present investigation fexhibited that the chosen isolates of *lactobacillus* are great probiotic strains, which concurred with previous study [18].

Cell viability assay

cytotoxicity was performed by utilizing MTT technique. Dose and time were depended on the cytotoxic impacts of *lactobacillus spp* against HeLa, RD and normal cell line (REF) cells and was carried out in three periods (24, 48 and 72 hrs. as showed in figure (2). Cancer cells viability was significantly inhibited according to time and dose treatment. It was shown that increasing of incubation time and dose of CFCS led to decrease the Hela and RD cancer cells viability and inhibition percentage were 48% and 36% respectively after 24hrs. of incubation. Then again, it was shown that in every incubation period and concentration that were used in this study, important inhibition effect on HeLa ($P<0.05$) was appeared, and the highest dose (70µg/mL) in the greatest time (72hrs.) gave the best inhibition effect against HeLa cells.

The antiproliferative impact of the CFCS was more noteworthy against HeLa than against RD cells. Additionally, the effect of **CFCS** against REF normal cells was determined , and it was indicated that CFCS had the important effect on cancer cells but no effect against REF normal cells. Therefore, over 90% of REF cells were viable . Production of pro-inflammatory cytokines, in addition to oxidative stress achieved by MTT was thought to have responsibility to cause cells death [19].

Apoptosis

The results showed that there is a significant effect of **CFCS** on cell proliferation in cancer cell lines (Hela and RD) and in normal cell line (REF) by using mitochondrial kit which was chosen to detect its antiapoptotic effects. The apoptotic cells appeared green regions and healthy cells were red regions. Both treated and untreated (control) cells have been delineated and computed under the fluorescent microscope using cationic fluorescence dye (mitocapture reagent). The percentage of apoptosis was determined as a mean of five fields for each test which was clearly showed apoptosis induction in treated groups in comparison to their control. The different concentrations of **CFCS** showed significant differences at $P \leq 0.05$ in comparison to control, and the high concentration 1000µg/ml gave 84% and 69% of apoptosis in Hela, and RD cells respectively. In living cells, mitochondria continuously divide and combine with each other [20] and Bcl-2 family members are engaged with these procedures. As a conclusion, it was discovered that **CFCS** has a cytotoxic activity, and confirmed that the tested isolates had anticancer activity impacts against some cancer cell lines (table 4**).**

Table (1): Result of Biochemical tests of *Lactobacillus spp*

Biochemical Tests	Result
Catalase test	–
Oxidase test	–
Indol test	–
Gelatinase test	–
Arginine test	+
Skim milk coagulation	+

Table 2: Antibiotic discs used in the present work

Id	Antibiotic discs	Disc potency(µg/ disc)	Zone diameter (mm)	Susceptibility
1	Vancomycin	30	21	S
2	Ceftazidime	10	16	I
3	Imipenem	10	23	S
4	Clindamycin	2	23	S
5	chloramphenicol	30	7	R
6	Erythromycin	15	25	S
7	Cefotaxime	10	16	I
8	Amoxicillin Clavulanic acid	10/20	18	S

S= sensitive ; R= resistant ; I= intermediate.

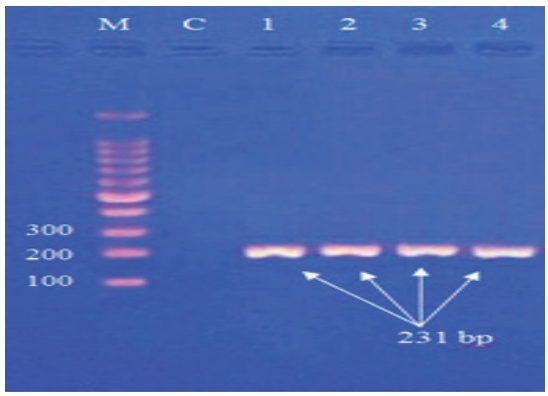
Table 3: The inhibitory effect of *Lactobacillus ssp* against pathogenic bacteria

Pathogenic bacteria	Growth	Susceptibility
Escherichia coli		ES
S. aureus		S
Streptococcus spp		I
P. aeruginosa		R
Candida albicans		R

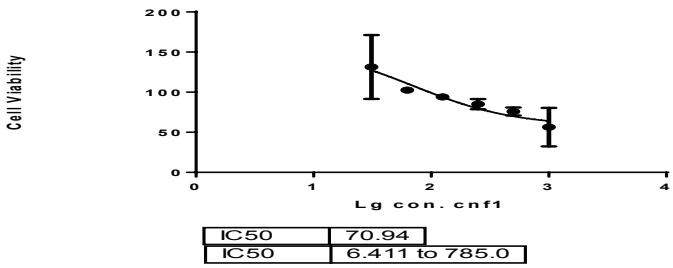
R=Resistant ,S=Sensitive (11-14) , Intermediate (6-10) ,ER=Extra sensitive >14

Table(4) Apoptosis ratio of cell lines induced by significant concentrations of CFCS

CFCS concentration(µg/ml)	Hela cell	RD cell	REF cell
1000	84	69	11%
500	78	61	6%
250	56	47	3%
125	46	32	0%



Figure(1) Gel electrophoresis for amplification genes encoded to 16SrRNA in Lactobacillus isolates. Electrophoresis was performed on 1% agarose gel and run with **at 5 V/cm for 1 hour, stained with ethidium bromide and visualized on a UV transiluminator documentation system. The size of amplified gene at 231pb**



Figure(2) Cytotoxicity effect of partial purified CFCS on Hela cells after 72 hours of incubation at 37ºC

Conclusion: The supernatant of *Lactobacillus* strains contained probiotic activity (antibacterial and anticancer) such as apoptosis in mitochondria.

Conflict of Interest: No conflict of Interest

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Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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