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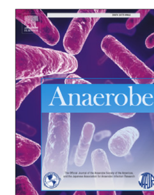

Clinical microbiology

Probiotic potential and biotherapeutic effects of newly isolated vaginal *Lactobacillus acidophilus* 36YL strain on cancer cells

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A B S T R A C T

Lactobacillus acidophilus is categorized as a probiotic strain because of its beneficial effects in human health and prevention of disease transmission. This study is aimed to characterize the probiotic potential of *L. acidophilus* 36YL originally isolated from the vagina of healthy and fertile Iranian women. The *L. acidophilus* 36YL strain was identified using 16S rDNA gene sequencing and characterized by biochemical methodologies, such as antibiotics susceptibility, antimicrobial activity, and acid and bile resistance. The bioactivity of the secretion of this strain on four human cancer cell lines (AGS, HeLa, MCF-7, and HT-29) and one normal cell line (HUVEC) was evaluated by cytotoxicity assay and apoptosis analysis. This newly isolated strain was found to exhibit notable probiotic properties, such as admirable antibiotic susceptibility, good antimicrobial activity, and favorable resistance to acid and bile salt. The results of bioactivity assessment demonstrated acceptable anticancer effects on the four tested cancer cell lines and negligible side effects on the assayed normal cell line. Our findings revealed that the anticancer effect of *L. acidophilus* 36YL strain secretions depends on the induction of apoptosis in cancer cells. *L. acidophilus* 36YL strain is considered as a nutraceutical alternative or a topical medication with a potential therapeutic index because of the absence of cytotoxicity to normal cells, but effective toxicity to cancer cell lines.

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1. Introduction

Specific lactic acid bacterial (LAB) strains, such as *Lactobacillus* strains, have been considered as probiotics because of their health benefits [1–4]. These strains have a long history of consumption in traditional fermented foods as natural inhabitants of healthy human gastrointestinal tracts. Probiotic bacteria are required to express high resistance to acid and bile, adhere to intestinal surfaces, and colonize in the gastrointestinal tract. Research findings have shown that several *Lactobacillus* bacterial strains possess the resistance to acid and bile, inhibitory activity toward the growth of pathogenic bacteria, and positive effects on the host health [5–8]. The key foundation for improving the functional properties and the biotherapeutic action of probiotic foods and pharmaceutical products is the selection of suitable probiotic candidates.

Cancer is one of the main causes of human deaths. Chemotherapy and chemoprevention are applied to control metastasis and decrease human mortality [9]. Studies have discovered that some specific strains of lactobacilli can induce the production of pro-inflammatory cytokines (interleukins IL-1 and IL-6) and anti-inflammatory cytokines (interleukins IL-12 and IL-10) in animal/human body. Evaluation of the toxicity of bacterial cytotoxic agents to different cell types is performed by in vitro cytotoxicity tests and by analyzing the mechanism of apoptosis in treated cancer cells [10]. Induction of apoptosis, which can be applied to control cancer development, involves complex anticancer activity of several therapeutic substances [11]. The HeLa cervical cancer cell line is a suitable model to study cervical cancer and apoptosis [12]. Apoptosis and necrosis are major forms of cell death that both

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implicate a sequence of successive biochemical and morphological events. Apoptosis or programmed cell death occurs during embryonic growth and in the course of organ involution. Internucleosomal DNA fragmentation and phosphatidylserine membrane translocation are crucial for the differentiation of necrosis and apoptosis [13]. Although various methods are available for identification, quantification, and characterization of apoptosis, flow cytometry is the most common choice to study apoptosis because of its applicability to an extensive range of cell types, stimulants, and time. Evaluating apoptosis by flow cytometry provides information on symptoms and incidence of apoptosis that is interpreted as a sign. Besides, cellular morphological characteristic is another method to determine the cell death mode (apoptosis or necrosis).

This study aims to characterize the probiotic potential of *Lactobacillus acidophilus* 36YL originally isolated from the vagina of healthy and fertile Iranian women. The acid and bile resistance, antibiotic susceptibility, and antimicrobial activity of this strain will be evaluated. Four different carcinoma cell lines will be used for testing the anticancer properties of *L. acidophilus* secretions by comparing results from paclitaxel treatment groups, as a positive control, and consequent induction of apoptotic cells through DAPI staining, DNA fragmentation, and flow cytometry which is broadly used to analyze the cytotoxicity.

2. Materials and methods

2.1. Sampling and isolation

The vaginal specimens obtained from 40 healthy and fertile Iranian women aged 17–36 years were evaluated for LAB isolation. The lateral vaginal wall was swabbed with sterile cotton-tipped applicators, which were stored in sterile carriers. The strain was isolated by inoculating the samples on de Man–Rogosa agar (MRS agar, Merck, Germany) supplemented with 0.2% bromocresol purple (Merck, Darmstadt, Germany) with streak plate technique and was incubated anaerobically at 37 °C for 48 h using an anaerobic jar. Identification was performed by phenotypic (colony and cell morphology) and molecular criteria, such as testing for Gram reaction, catalase activity [14], and 16S rDNA sequencing.

2.2. Genomic DNA extraction and amplification of 16S rDNA region

The total DNA of isolate from the inoculated culture with a single colony was extracted using the procedure described previously by Cardinal [15]. A single colony was recultured in MRS broth at 37 °C for 24 h, and 1.5 ml of the bacterial culture was then centrifuged at 10,000× g for 5 .The supernatant was discarded, and the pellet was used for DNA isolation. The extracted DNA was then suspended in 50 μl distilled water. The solved DNA sample was checked qualitatively and quantitatively by 0.8% agarose gel electrophoresis and spectrophotometry.

The extracted genomic DNA of the isolate was subjected to PCR analysis. Amplification of 16S rDNA region was performed in a thermal cycler MWG BIOTECH (Galileo, Madrid, Spain) using a pair of LAB-specific universal primers (Hal6F/Hal6R) [16]. PCR amplification was performed with an initial denaturation at 94 °C for 4 min, followed by 32 cycles; denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min [17].

2.3. 16S rDNA gene sequencing

PCR products from the 16S rDNA gene (1500 bp) were amplified using the Hal primer set. The sequencing of purified PCR product was conducted by Macrogen DNA Sequencing Service (Korea). Sequence similarity was determined via GenBank BLAST analysis [18].

2.4. Resistance to low pH and tolerance to bile-salt condition

To determine the isolate resistance for gastric acid and high bile salt conditions, a previous method was modified and employed [19]. Bacterial cells from overnight culture were harvested after centrifugation at 10,000× g at 4 °C for 7 min and washed twice with PBS buffer (pH 7.2). The washed cells were respectively placed in sterile tubes at pH 3.0 for different time points (0, 1, 2, 3 h) and 0.3% (w/v) bile salts (oxgall) for 0, 1, 2, 3, and 4 h before reculturing on MRS agar. Resistance was measured in triplicate in terms of viable colony counts and enumerated on MRS agar after incubation at 37 °C overnight.

2.5. Antimicrobial activity assay

Several pathogenic organisms were selected from the Persian Type Culture Collection to detect the antagonistic substances (Table 2). Well diffusion technique was used to detect the production of inhibitory substances in the supernatant fluids of the isolates. An overnight culture of the indicator strains was applied to inoculate the appropriate agar growth media [20] at 37 °C. The wells (diameter, 5 mm) were cut into agar plates, and 50 μl of filtered cell-free supernatant fluid obtained from the third subculture of the microorganisms grown in MRS broth were added to each well. The supernatant was obtained by growing the inhibitory producer strains overnight in MRS broth at 37 °C. The cells were removed by centrifugation, and the supernatant were placed in the wells and allowed to diffuse into the agar at room temperature for 2 h. Consequently, the plates were incubated at optimum growth temperature of the indicator strains and were examined after 24 h to measure the diameter of the inhibition zone areola [21,22]. The inhibition zones around the wells were measured and results were expressed in terms of resistance (0 mm), moderate resistance (0–4 mm), moderate susceptibility (4–8 mm), susceptibility (8–12 mm) and extra susceptibility (>12 mm) by comparing with performance standards for antimicrobial susceptibility testing.

2.6. Determination of antibiotic resistance

The antibiotic susceptibility of this strain was studied against the commonly prescribed antibiotics to common diseases (e.g., gentamycin, penicillin, tetracycline, ampicillin, erythromycin, vancomycin, clindamycin, chloramphenicol, and sulfamethoxazol) using disc diffusion method [23]. The isolate diluted culture (100 μl; approximately 10^6–10^7 viable cells) was diffused onto the Mueller Hinton Agar, and antibiotic discs were applied onto the surface using an antibiotic disc dispenser. The plates were incubated at 37 °C under anaerobic conditions and were assessed after 24 h of

Table 1
Survival rate of vaginal *Lactobacillus acidophilus* after incubation at pH value 3.0 and bile salts 0.3%.

Strain	SR%	Final counts (log cfu/ml) after incubation at:				
		0 h	1 h	2 h	3 h	4 h
Lactobacillus acidophilus[a]	81	9.91	8.83	8.45	8.02	–
Lactobacillus acidophilus[b]	89	8.38	7.81	7.68	7.54	7.46

[a] Survival rate after 3 h in low pH value 3.0.
[b] Survival rate after 4 h in bile-salt condition (0.3%).

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