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In vitro antagonistic effects of *Lactobacillus acidophilus* PTCC 1643 (DSM 20079) and *Lactobacillus plantarum* PTCC 1058 (ATCC 8014) against isolated bacteria from Urinary Tract Infections

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ABSTRACT

Lactic acid bacteria (LAB) are gram positive and non- spore forming bacteria that can produce some special substances for growth inhibition of pathogenic, non-pathogenic and food spoilage bacteria. The aim of this study was to determine the inhibitory effects of *Lactobacillus acidophilus* PTCC1643 (DSM 20079) and *Lactobacillus plantarum* PTCC 1058 (ATCC 8014) cultural supernatants against urinary tract infections (UTI) agents, including *Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli* and *Lactobacilli* spp in MRS Broth. Selective culture media and biochemical tests were used for UTI bacteria isolation. Inhibitory effects were evaluated using well diffusion agar and the inhibitory zone diameters were measured after overnight incubation at 35-37°C. The highest inhibitory effect were obtained using *L. plantarum* PTCC 1058 cultural supernatant against *E.coli* and the lowest one using *L.acidophilus* PTCC 1643 cultural supernatant against *K.pneumoniae*. These *Lactobacilli* spp.were showed inhibitory effects on all of the isolated bacteria. Totally, *L.plantarum* PTCC 1058 showed inhibitory activity more than *L.acidophilus* PTCC 1058. The findings of this study showed that *Lactobacilli* spp. as a capable candidate for producing antibacterial compounds and using them for treatment and industrial usage.

1. Introduction

Lactic acid bacteria (LAB) are a group of gram positive, non spore forming, acid tolerant, catalane negative rod bacteria with a low G+C content that play a vital role in our life, due to their antimicrobial action (Kleerebezem et al., 2010; Sieladie et al., 2011; Rattanachai kunsopon et al., 2010; Saranya et al., 2011). Lactic acid bacteria generally recognized as safe (GRAS) organisms (Sieladie et al., 2011; Patrick, 2012). These micro–organisms are widely used in the food industry as starter culture under controlled conditions for fermentation, especially for dairy products (Sieladie et al., 2011; Sankar et al., 2012; Yang et al., 2012). Some of them are an important part of normal flora in our body and found in the mouth, gastrointestinal tract and female genitourinary tract (Selvamohan et al., 2010). Lactic acid bacteria produce various compounds such as organic acids, dactyl, hydrogen

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peroxide, D isomers of amino acids, Co_2, acetaldehyde and bacteriocins or bactericidal proteins during lactic fermentations (Saranya et al., 2011; Yang et al., 2012; Rattanachai kunsopon et al., 2010; Saranya et al., 2011).

Among these compounds, proteinaceous bacteriocins have gained much attention especially regarding their role in the dairy foods where they are known to strongly inhibit the growth of pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella*, *Shigella*, and *Helicobacter* (Saranya et al., 2011; Dardir et al., 2012; Rattanachai kunsopon et al., 2010). So, bacteriocins are ribosomal synthesized antimicrobial compounds that are active against other bacteria, either of the same species or across genera (Rattanachaikunsopon et al., 2010; Yang et al., 2012).

Urinary tract infections (UTI), as the second most common type of infections, are serious health problems (Mittal et al., 2009; Tanvir et al., 2012). It can be associated with the substantial morbidity and significant expenditures in persons of all ages (Biadglegne et al., 2009; Adeniyi et al., 2006). The urinary tract is normally sterile, but the bacteria can migrate to the urethra from the rectum or vagina (Mohsin et al., 2010; Kathleen et al., 2008). Also, the most urinary tract infections agents are both gram positive and gram negative bacteria. *E.coli* remains the leading cause in 75% to 90% of the cases (Kathleen et al., 2008; Tanvir et al., 2012). UTI caused by other gram negative bacilli including *Klebsiella pneumonia*e, *Proteus mirabilis* and *Enterobacter aerogenes* and gram positive coccid such as *Staphylococcus saprophyticus*, *Enterococci*, *Streptococcus faecalis* and *Staphylococcus aureus* too*(*Kathleen et al., 2008; Marelli et al., 2004*)*.

While antibiotics are used to treat and prevent recurrent urinary tract infections, but they are the main weapon against infection as using of antimicrobial agents is not only select resistance bacteria but it can disturb the balance of body by killing the friendly bacteria (Kathleen et al., 2008; Selvamohan et al., 2010; Tanvir et al., 2012). For this reason, probiotics have been suggested as an alternative therapy for the treatment of urinary tract infections (Carson et al., 2003). Probiotics are defined as living microorganisms which confer a health benefit on the host (Pishva et al., 2009; Sanders et al., 2009; Selvamohan et al., 2010). Most probiotics are strains of *Bifidobacterium* or *Lactobacillus species* (Pishva et al., 2009). The probotic effects of these organisms include the treatment of various types of diarrhea, alleviation of Crown's disease and balancing of intestinal micro flora through the growth modulation of bacteria present in the gastrointestinal tract, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection the other physiological benefits of probiotics are anti carcinogenic activities, lowering of cholesterol, immune stimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, improvement of lactose utilization and protection against other diseases (Pasha et al., 2009; Sarnia et al., 2011; Adenitis et al., 2006). The main goal of this study was to determine the inhibitory effects of *Lactobacillus acidophilus* PTCC 1693 and *Lactobacillus plantarum* PTCC 1058 against UTI agents in different pH.

2. Materials and Methods

2.1. Preparation of standard strains

Lactobacillus acidophilus PTCC 1693 and *Lactobacillus plantarum* PTCC 1058 were purchased from the microbial collection center of the Iranian Scientific and industrial researches organization. They were activated at DeMan, Rogosaand Sharp medium (MRS Broth) and incubated under anaerobic conditions at 37°C for 18- 24h by using a candle jar with a moistened filter paper to provide a Co_2 enriched, water vapor saturated atmosphere. After preparation of suspension, it was prepared in 0.5 McFarland concentrations, cultured *Lactobacilli* was centrifuged at 3000 rpm for 10 min and then the supernatant was adjusted to pH 6.5 – 7 with1 N NaOH.

2.2. Isolation and identification of bacteria from urine samples

Urine samples were collected from three hospitals (Rasht– Iran) in sterile plastic universal containers and transported to the laboratory in an ice cold condition. For isolation of UTI causing organisms, loop full of urine samples was streaked on blood agar, MacConkey agar and EMB agar plate and incubated at 37°C for 24h. After incubation, the colonies were selected and characterized on the basis of morphological, cultural and biochemical tests and were identified according to Bergey's Manual of Systematic Bacteriology (Bergey's manual of determinative bacteriology).

2.3. In vitro Antagonistic activity

In this study well diffusion method was used. In this method 100 µl of isolated pathogens with 0.5 McFarland concentrations were swabbed on the Mueller Hinton agar plates. Then wells were punched over the agar plates by using sterile gel puncher. After 100 µl of each culture supernatants of lactobacilli were transported to the wells and plates were incubated at 37°C for 24h. Then the inhibitory activity was determined by measuring the inhibition zone around each well and expressed in millimeter.

2.4. Effect of pH

The pH of supernatant was adjusted to 4, 6, and 8 and then kept at room temperature for 4h. Then100 µl of isolated pathogens with 0/5 McFarland concentration, were swabbed on the Mueller Hinton agar plates, and the wells were punched over the agar plates by using sterile gel puncher. Then, 100 µl of each culture supernatants samples treated at different pH values were transported to the wells and the plates were incubated at 37°C for 24h. There after, the inhibitory activity was determined by measuring the inhibition zone around each well and expressed in millimeter.

3. Results

Antimicrobial activity of bacteriocin produced by *Lactobacillus acidophilus*PTCC (DSM 20079) and *Lactobacillus plantarum* PTCC 1058 (ATCC 8014) against indicator bacteria after incubated at 37°C for 24h has been studied.

The largest zone of inhibition was produced by *L.plantarum* (21.63 mm) against *E.coli* while the smallest zone of inhibition was produced by *L.acidophilus* (13.65mm) against *K.pneumonia*e (Figure 1).

In the present study, the activity of supernatant was found in various pH ranges. The maximum zone of inhibition produced by *L.plantarum* at pH 6(24.6) against *E.coli* while the least zone of inhibition (7.5 mm) produced by *L.acidophilus* at pH 8 against *K. pneumonia* (Figures 2, 3).

4. Discussion

In recent years, there has been an increased focus on the use of probiotic such as *Lactobacillus* sp. because of the numerous benefits including the treating of asymptomatic bacterial vaginosis, hypercholesterolemia, irritable bowel disease, cardiac diseases, artherosclerosis, arteriosclerosis, anti–tumor effect and treatment of urinary tract infection (Bhadoria et al., 2011; Sieladie et al., 2011; Selvamohan et al., 2010).

Probiotics are live microorganisms present in the food and dietary supplements that beneficially affect the individual by improving the intestinal microbial balance properties and inhibition the growth and proliferation of some pathogens with specific mechanism such as production of inhibitory compounds like bacteriocins (Adeniyi et al., 2006; Dixit et al., 2013; Savadogo et al., 2006). The bacteriocins produced by gram positive bacteria like LAB, are small peptides that are used as preserve daily foods and treatment of different diseases (Saranya et al., 2012; Savadogo et al., 2006). Lactobacilli are highly competitive largely due to their applications in the production of fermented food. They can also produce antimicrobial substances including bacteriocins that have the ability to inhibit pathogenic and food spoilage bacteria (Rattanachai kunsopon et al., 2010).

It has been reported that *Lactobacillus acidophilus* is a well-known and well-studied probiotic microorganism. However, it is now clear that different stains undoubtedly vary in their efficiency and probiotic potentials (Dixit et al., 2013). The treatment of UTI with LAB has been shown in many studies (Reid and Seidenfeld, 1997; Reid et al., 2003). The largest zone of inhibition was produced by *L.lactis* K_3 (15mm) against *S.saprophyticus* (UCH 2051) (Adeniyi et al., 2006). Selvamohan and Sujitha in 2010 reported the antimicrobial activity of bacteriocin produced by *L.plantarum* that the highest inhibitory activity (22mm) was against *E.coli* while the least activity (10mm) was against *Streptococcus* sp. (Selvamohan et al., 2010). Similar study was reported by Hemashenpagam in 2011, who studied the activity of LAB on some gram positive and gram negative pathogenic bacteria that the largest zone of inhibition was produced by *L.plantarum* (25mm) against *S.aureus* and the least zone of inhibition was 5mm (Saranya et al., 2011). In this study *L.plantarum* PTCC 1058 (ATCC 8014) showed the inhibitory activity more than *L.acidophilus* PTCC 1058 (DSM 20079) against the indicator bacteria and the highest inhibitory effect were obtained using *L.plantarum* PTCC 1058 cultural supernatant against *E.coli.*

It was reported that the LAB antimicrobial compounds (*L.fermentum, L.casei, L.acidophilus, L.lactics*) have the bactericidal effect against gram positive and negative pathogenic bacteria (Conconnire et al., 1998).

It was shown that the antimicrobial activity of bacteriocin produced by *L.plantarum* that the highest inhibitory activity (25mm) was against *E.coli* at pH 6 and this inhibitory activity at this pH about *Streptococcus* sp. was (7.5mm). They reported the largest zone of inhibition was produced by *L.plantarum* against *E.coli* (Selvamohan et al., 2010). This is in agreement with our work. Different reports showed that most lactobacilli strains produce substances that inhibit pathogenic, non–pathogenic and spoilage organisms in fermenting foods and beverage. Ingeneral, the antimicrobial activity of lactobacilli may be due to organic acids, hydrogen peroxide, bacteriocins or other inhibitory substances from metabolites (kuwaki et al., 2010).

In vitro inhibitory effects of LAB showed the largest zone of inhibition by *L.acidophilus* against *E.coli*. (Darter et al., 2012). The diameter zone of inhibition *E.coli* O:157 H:7 was 30-45 mm and for *Salmonella typhus* was 18-32mm (Pasha et al., 2009). Perusal of data pertaining of the antimicrobial activity of *L.acidophilus* cultures against some common intestinal pathogenic organisms indicated that all the cultures of *L.acidophilus* were active against the tested intestinal pathogenic organisms. The range of inhibition zone of the tested pathogenic organisms was from 10.5–16.25 mm in diameter (Padmanabha et al., 2006). Ogunbanwo et al, reported the largest zone of inhibition produced by *L.brevis* and *L.plantarum* against *Bacillus cereus* (8-10mm) and the other result against *E.coli* and *Yersinia enterocolitica* was 6-8mm and 6-7mm,respectively (Ogunbanwo et al., 2003). Use of cultural supernatant of *L.fermentum, L.casei. L.lactis* and *L.acidophilus* have antagonistic effects on pathogenic bacteria (Coconnier et al, 1998).The supernatants of *L.curvaus* and *L.plantarum* had inhibitory effects with using well diffusion method against *S.aureus*, *Yersinia enterocolitica*, and *Listeria monocytogenes* (Hirano et al., 2003). More studies showed probotic products of LAB had inhibitory effects against gram positive and negative pathogenic microorganisms. *Lactobacillus* spp. were showed inhibitory effects on all of the isolated bacteria from urinary tract infections; Totally, *L.plantarum* PTCC 1058 showed inhibitory activity more than *L.acidophilus* PTCC 1058.

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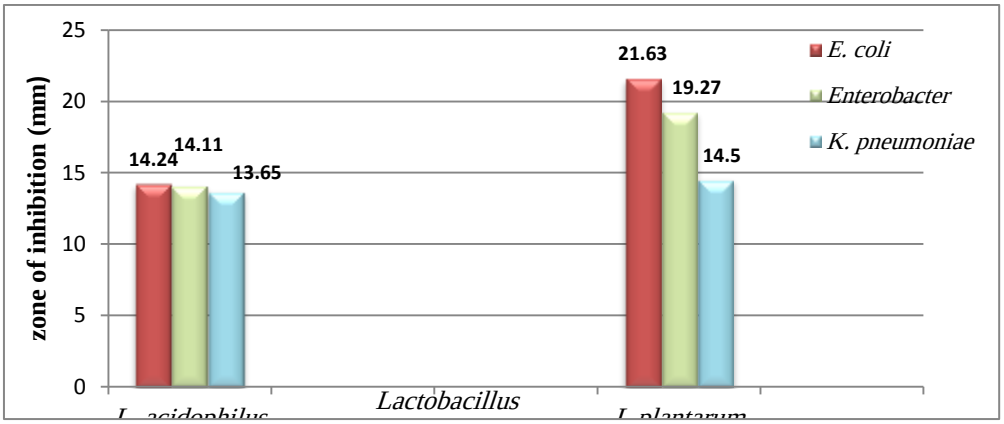

Figure 1. Inhibitory effects of *L.acidophilus* PTCC 1693 and *L.plantarum* PTCC 1058 against urinary tract infections (UTI) agents at 37°C for 24h.

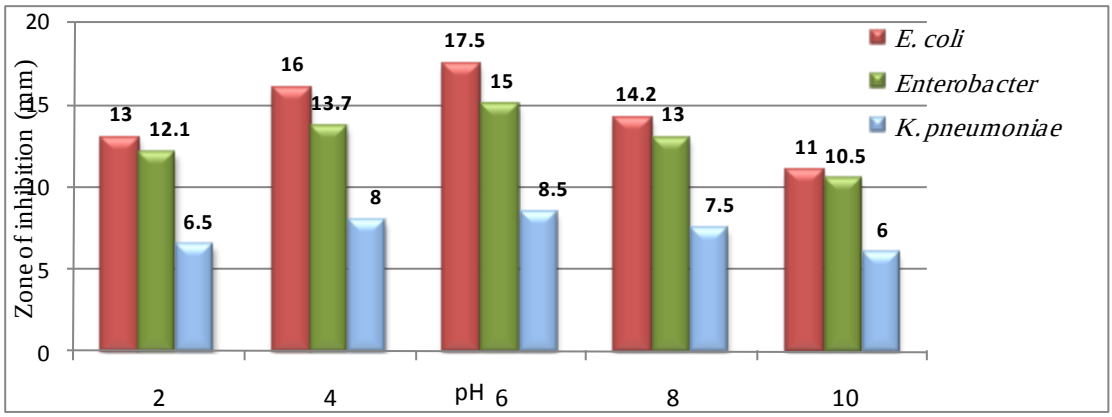

Figure 2. inhibitory effects of *L.acidophilus* PTCC 1693 against urinary tract infections (UTI) agents in different pH.

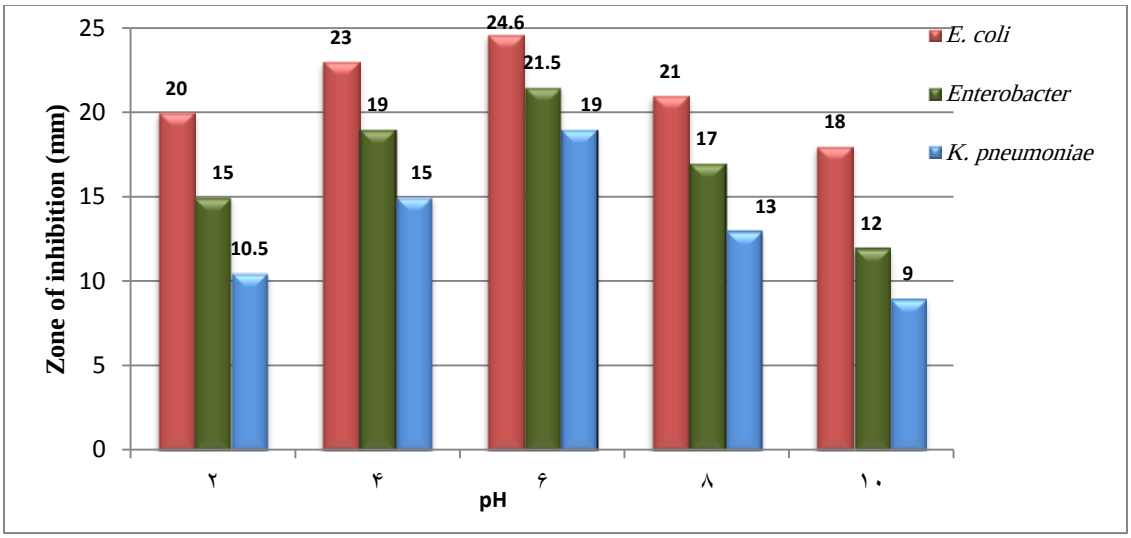

Figure 3. Inhibitory effects of *L.plantarum* PTCC 1058 against UTI agents in different pH.

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