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Lactobacillus plantarum LPS10 as probiotic for prevention urogenital bacterial infections

Soheir S Abdel Salam[1]* , Gamal Enan[2] , and Hytham M. Ahmed[3].

[1]Department of Microbiology, Faculty of Science, Benha University.
[2]Professor of Microbiology, Botany Department, Faculty of Science, Zagazig University.
[3]Candidate for Master Degree, Faculty of Science, Benha University.

ABSTRACT

Bacteria are considered one of the most frequent injuries infecting both male and female urinary tract (UTI). The multi-drug resistance phenomenon in bacteria leads to the necessity of finding an alternative remedy. Probiotics are suggested as a suitable and appropriate solution. In this study, cell free supernatant (CFS) of *Lactobacillus plantarum LPS10* is examined for its inhibitory effect against clinical urogenital bacterial strains and *Candida albicans* isolated from UTI infections. The growth of *Staph. aureus* after 24 hours of incubation and the growth of *E. coli* after 48h of incubation could inhibit by e CFS of this probiotic bacteria. The growth of other pathogens also decreased at different times of incubation. This reflects the ability to use *L. plantarum LPS10* as a probiotic. CFS obtained from this probiotic bacteria showed a promising antibacterial and anticandidial results.

Keywords: Urogenital infections, Probiotics, multi-drug resistance, inhibitory effect

Corresponding author

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INTRODUCTION

UTIs are the most common bacterial infection and have a significant societal and economic burden **(1).**Clinical syndromes include: dysuria, frequency, suprapubic tenderness in cystitis and urethritis, flank pain, fever, and urgency in pyelonephritis(**2)**.The emergence of antibiotic resistance in the management of UTIs is considered a danger threatens man life especially in the undeveloped countries where poverty and ignorance prevail. Moreover, no hygienic rules or customs are taken into account**(3).**

Probiotics (derived from Latin and Greek) means "for life" is defined according to **FAO/WHO** as: Live microorganisms which when administered in adequate amounts confer a health benefit on the host.

Lactobacilli are gram-positive rods, facultative or strict anaerobes that have a fastidious growth requirement. They prefer an acidic environment and can help creating one by producing lactic acid and other acids. Lactobacilli have been regarded for long decades to be safe and regarded as nonpathogenic members of the intestinal and urogenital floras **(4)**.These lactobacillus strains possess the criteria of adherence and colonization to tissues and the possibility to inhibit the pathogenesis of urogenital pathogens. So, they are considered as effective probiotic agents **(5)**.

Lactobacillus plantarum LPS10, a gram-positive, rod- shaped bacterium that can be considered as a valuable probiotic to biocontrol of urinary tract pathogens. The inhibitory activity of *Lactobacillus plantarum* could be due to lactic acid, diacetyl, CO_2, acetaldehyde or H_2O_2**(6,7,8)**.The LPS10 organism produces protease, β-glucosidase, amylase and esterase and, consequently, considered ideal probiotic and these enzymatic activities are qualified properties for any organism to be used as probiotic bacterium **(11)**. Oxalate, citrate are utilized by the LPS10 organism and acetoin is also produced by the LPS10 organism **(12)**. For the above reasons,the LPS10 lactic acid bacterium could be used as protective and probiotic bacterium.

Aim of the work: Urogenital infections are a serious problem that may face all human beings during the span of their lives. In this study, we aimed to control the clinical bacteria isolated from patients suffering from urinary tract infections by *Lactobacillus plantarum LPS10*.

MATERIALS AND METHODS

Bacterial strains and culture media: The probiotic bacterium *L.plantarum LPS10* was kindly provided by Prof. Dr. Gamal Enan, Faculty of Science, and Zagazig University. It was propagated and subcultured on De Man, Rogosa and Sharpe (MRS, Oxoid) medium. It produced inhibitory activity as described by **(13)**. The inhibitory substances were due to acids and bacteriocins which designated as plantaracin.

The sensitive microorganisms used were isolated from urogenital patients admitted to Fakous general hospital, Al-Sharkia, Egypt.Four Bacterial isolates were obtained from both males and females at different ages. Four isolates were obtained and were designated *P. aeruginosa*(1U), *S.aureus* (2U), *K. pneumonia* (4U) and*E. coli* (6U). Another four bacterial isolates of urogenital origin were kindly provided by Prof. Dr. Ahmed Anwar Shahin from Faculty of Medicine, AL- Qasr Al- Aini. They were also designated, *Shigella* (31U), *C. albicans* (32U), *Salmonella paratyphiB* (33U) and *Proteus vulgaris* (34U).

Preparation of cell free supernatant (CFS):

L. plantarum LPS10, the producer of inhibitory substances, as grown in De Man, Rogosa and Sharpe broth for 16h at 37°C. CFS was obtained by centrifuging the culture (10.000 x g for 15 min at 4 °C) then the supernatant was collected.

Inhibition test of microbial pathogens by L. plantarum LPS10 CFS:

Erlenmeyer flasks of 250 ml, each containing 100 ml BHI broth, were inoculated by 2.0x 10^4 CFU/ml of *L. plantarum LPS10* and about 2.0x 10^4 CFU/ml of each bacterial pathogen used. In another experiment MRS broth inoculated by *L. plantarum LPS10* (by 2.0x 10^4 CFU/ml) was inoculated also by about by 2.0x 10^4 CFU/ml of the C. albicans. Another series of 250 ml Erlenmeyer flasks, each containing 100 ml Brain Heart Infusion (BHI) broth, were inoculated either by 2.0x 10^4 CFU/ml of each bacterial pathogen or by 2.0x 10^4 CFU/ml of*C.*

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albicans used; and was treated by 1% v/v of CFS. The inoculated and treated containing both bacterial samples and controls were incubated at 37°C for 3 days or 6 days (Candidal test). After suitable time intervals 1 ml aliquots were removed, serially diluted and plated on to agar plates containing the selective and specific agar media for enumeration of each microbe used (Oxoid). These specific agar media were inoculated by sample dilutions and incubated at 37°C for 48h. Then CFU/ml was obtained as described previously for similar experimental conditions **(14, 15)**.

RESULTS AND DISCUSSION

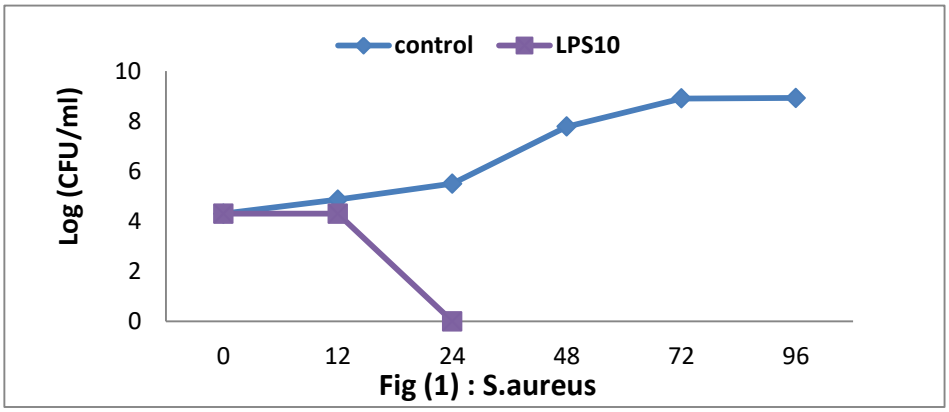
It was, preliminary, necessary to test the susceptibility of uropathogens to different classes of antibiotics. Results are given in table (1).

Table (1): Antibiotic susceptibility profile of selected clinical bacterial isolates.

bacterial isolates	Tested antibiotics												
	IPM	AK	CRO	RF	CTX	AZM	FEP	CFP	CN	AMC	E	OFX	LEV
1U	R	R	R	R	R	R	R	R	R	R	R	R	R
2U	S	I	I	R	R	R	R	R	R	R	R	R	R
4U	S	I	R	R	R	R	R	R	R	R	R	R	R
6U	S	R	R	R	R	R	R	R	R	R	R	R	R
31U	S	I	R	R	R	R	R	R	S	R	R	R	R
32U	R	R	R	R	R	R	R	R	R	R	R	R	R
33U	S	R	S	I	R	R	R	R	R	R	R	R	R
34U	R	S	I	R	R	R	R	R	S	I	R	R	R

From the above table, it was shown that all the bacterial isolates except *P. vulgaris* (isolate no 34U) were nearly resistant to all antibiotics excerpt for Imipenem (IPM) while *P. vulgaris* was sensitive for Gentamicin (CN) and Amikacin (AK).The table illustrates that *C. albicans* (isolate no 32U) is completely resistant to all antibacterial agents. These were interesting results and encourage us to check and evaluate the inhibition of such urogenital bacterial pathogens by theprobiotic bacterium *L. plantarum LPS10*.

The study was further conducted to evaluate antibacterial activity CFS of *L. plantarum LPS10* against the 8 urogenital pathogens. Results are given in (fig. 1-8). Urogenital pathogens alone in control experiments grew vigorously and viable cells recorded 4-5 log cycles increase within 96h. In samples treated with CFS of *L. plantarum LPS10* thoroughly with urogenital pathogens, viable growth of urogenital bacterial pathogens was completely inhibited after 24h and 48h as in case of *S. aureus* (isolate no. 2U) and *E. coli* (isolate no. 6U) respectively. In the rest uropathogens, the viable growth decreased by 2log cycles within 96h. Almost the difference in colony counts (CFU/ml) between the control samples and treated ones with CFS of *LPS10* was 4-5 log cycles within 96h. This is reflecting the strong inhibitory effect of L. plantarum LPS10 as a probiotic.



Fig(1): Effect of CFS of *L. plantarum LPS10* on *S. aureus* (isolate 2U).

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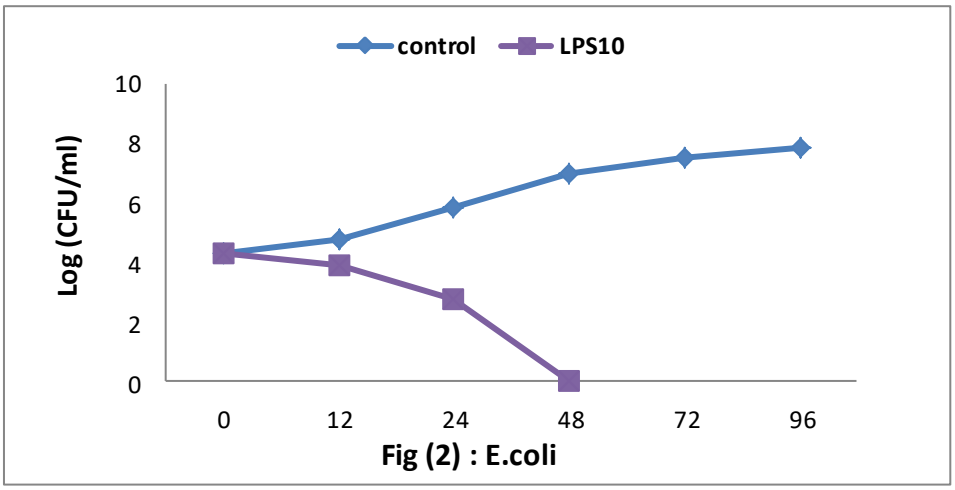

Fig (2): Effect of CFS of *L. plantarum LPS10* on *E. coli* (isolate 6U).

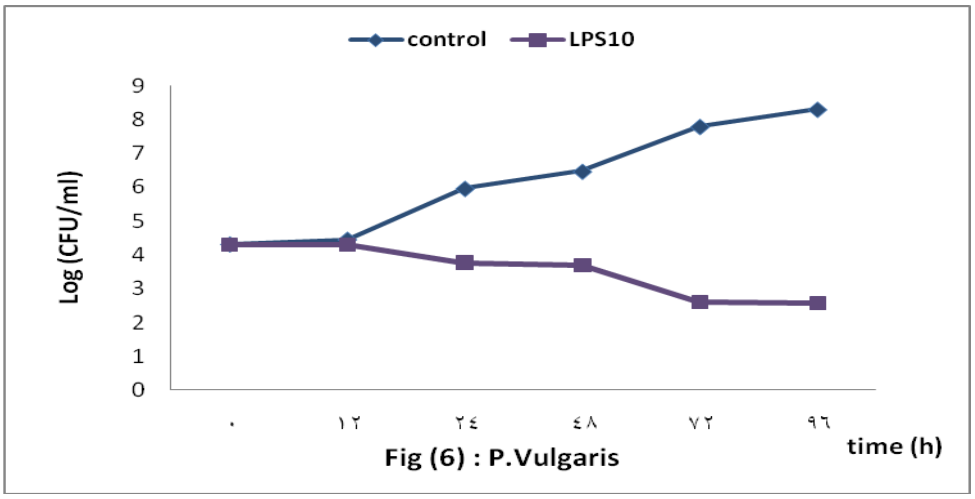

Fig (3): Inhibition of *P. aeruginosa* (code 1U) by CF S of *L.plantarum LPS10*

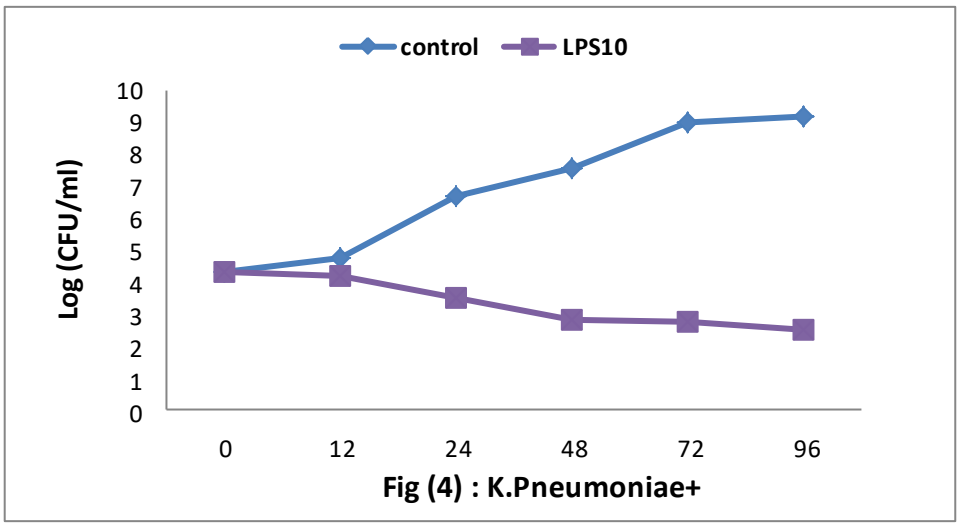

Fig (4): Inhibition of *k. Pneumonia* (code 4U) by CFS of *L. Plantarum LPS10*.

ISSN: 0975-8585

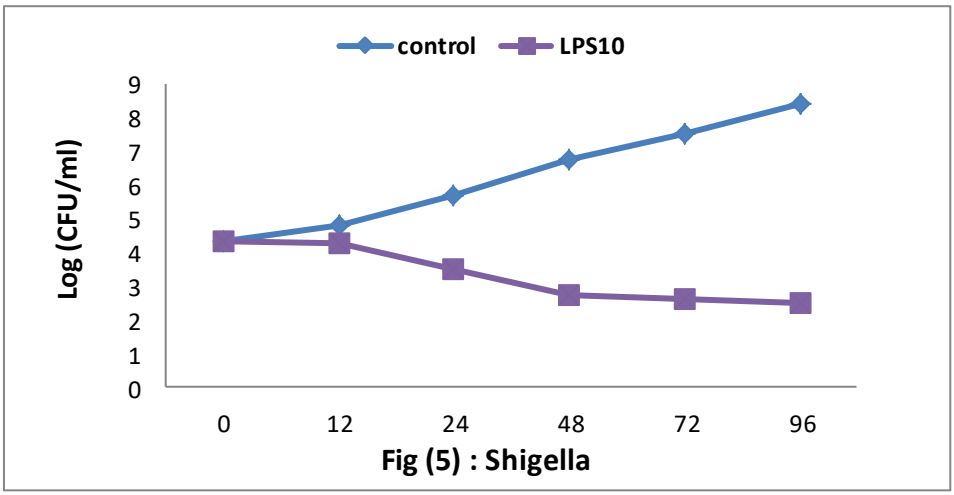

Fig (5): Inhibition of *Shigella* (code 31U) by CFS of *L.plantarum LPS10*.

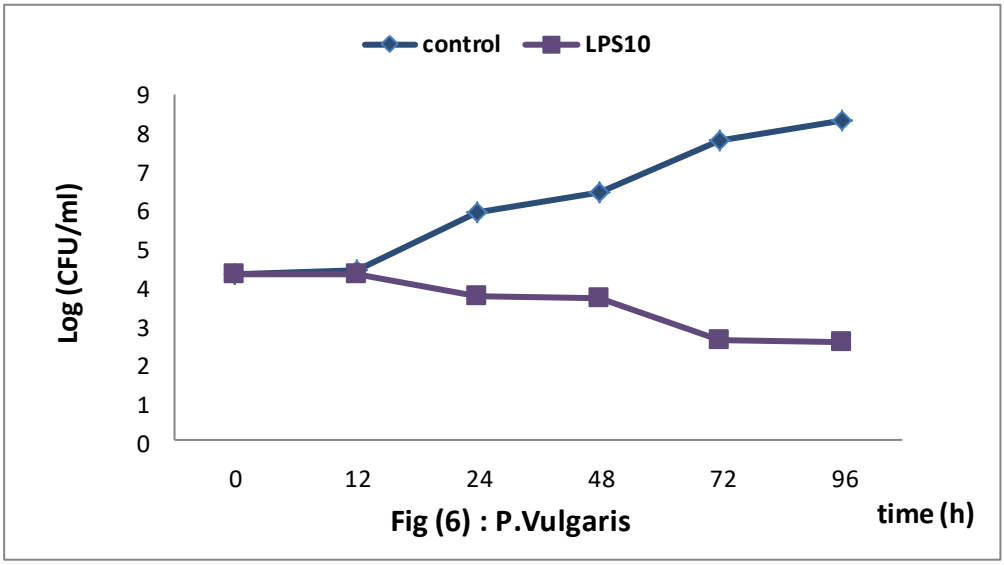

Fig (6): Inhibition of *P. vulgaris* (code 34U) by CFS of both *L.plantarum LPS10*.

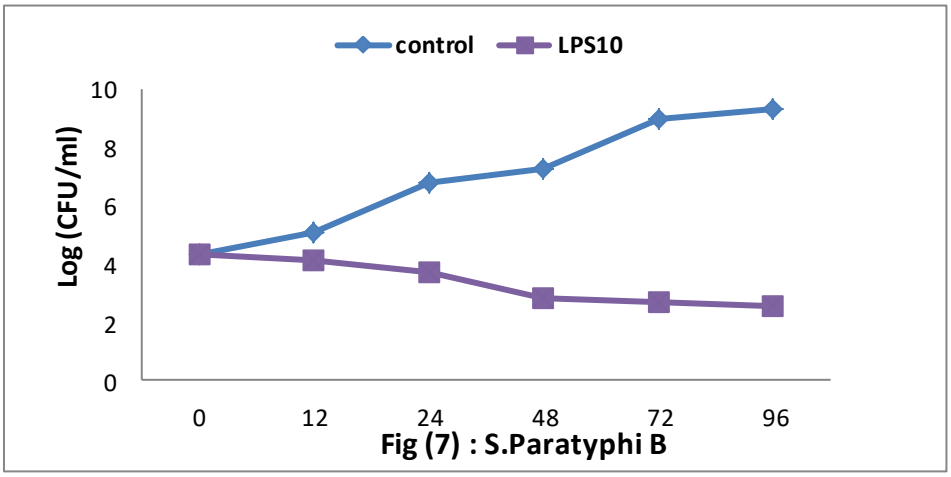

Fig (7): Inhibition of *S. paratyphi B* (code 33U) by CFS of *L. plantarum LPS10.*

ISSN: 0975-8585

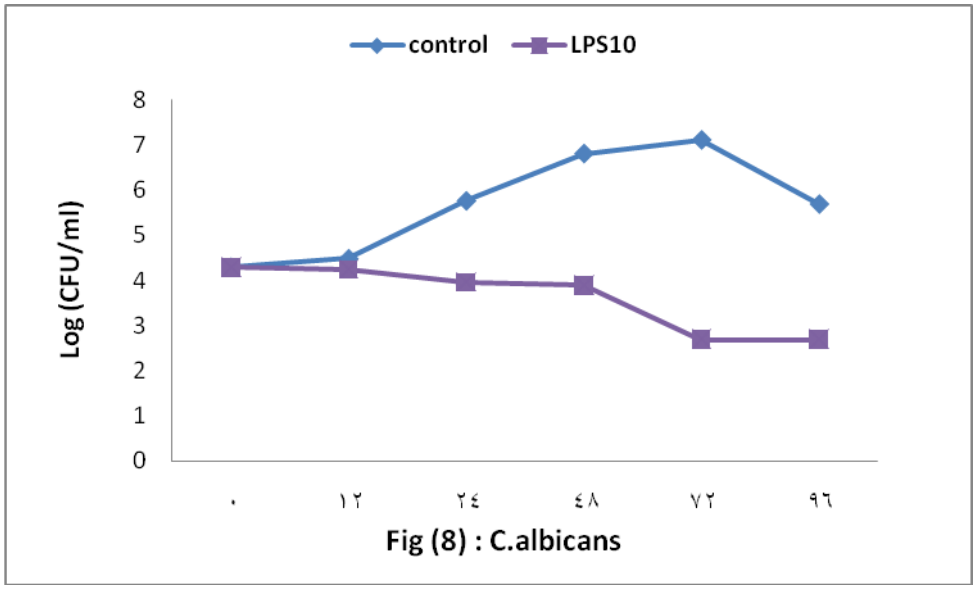

Fig (8): Inhibition of *C. albicans* (code 32U) by CFS of*L.plantarum LPS10*.

The effect of CFS of *LPS10* on *C. albicans* (isolate no. 32U) was also examined. The former could decrease the growth of the viable cells of the latter by 2 log cycles within 96h. Counts of *C. albicans* were carried out on Sabouraud agar. In control experiment, *C. albicans* cells increased from 20x 10^4 to 50x 10^8 within 96h. However, in samples treated with *LPS10*, viable cells decreased by 2 log cycles within 4 days. Urinary tract infections are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings and ranks high amongst the most common reasons that face an individual to seek medical attention **(16, 17, 18, and 19)**. UTIs encompass a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis **(20, 21).** It represents one of the most common diseases encountered in medical practice today, affecting people of all ages, from the neonate, adolescence to the geriatric age group **(22)**. Most infections are caused by retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney especially in the females who have a susceptibility to be infected due to their shorter and wider urethra and are more readily transversed by microorganisms **(3)**.

Although fungi and viruses are occasional etiological agents, UTIs are predominantly caused by bacteria. The most common bacteria implicated as causative agents of UTI generally originate in the intestine and include but not limited to *E. coli, Pseudomonas spp, streptococcus spp, Neisseria gonorrhea, Chlamydia trachomatis, Candida spp, Proteus spp, Klebsiella spp, Mycoplasma***(18).**

As antimicrobial treatment of UTIs is not always effective, and bacteriuria remains due to bacterial and yeast resistance, recurrent infections **(23, 24)**, as well as side effects,it is no surprise that alternative remedies are of great interest to patients and their caregivers.

Probiotic bacteria, such as *Lactobacillus* strains are most commonly group of microorganisms used for treatment of many infectious diseases in human beings. These bacteria are well-known because they contain many beneficial properties to control pathogenic microorganism's ability. These properties include adherence to cells, reduction of pathogenic bacterial adherents and co-aggregation, and production of organic acids which antagonize pathogenic microorganisms and in the same time they are nonpathogenic **(25,26)**. In addition, there have been many reports on production of bacteriocin by *Lactobacilli* bacteria **(27, 28)**. It is known that the antagonistic activity of such bacteria is to inhibit a large number of enteric and urinary tract bacterial pathogens **(29, 30)**.

In this study, the indicator bacteria were isolated from urine samples of urogenital patients. The control of these uropathogens is necessary. The antibiotic susceptibility test was carried out. The general percentage of resistance of bacterial pathogens against the antibiotic discs used was high. This agrees with previous studies in this concern **(31)**. The *L. plantarum* LPS10 strain grew well in MRS broth and acidified the

medium rapidly and final pH reached 3.6 after 12h, produced protease, esterase, amylase and glucosidase and hence could be used as probiotic. The production of plantaracin was optimum at initial pH 6.5 and at incubation temperature of about 35°C when the producer organism was in the mid to the late exponential phase of growth (**32)**. Due to the last reasons, *L. plantarum LPS10* could be used as an ideal probiotic. It was reported that some criteria should be available in a bacterial organism to be used as a probiotic such as production of inhibitory substances as proteins or acids, production of H_2O_2, adherence to genital surfaces. L. plantarum LPS10 proved to have most of these features; consequently can be used as a probiotic to control urogenital pathogens. The urogenital probiotics can be administered orally or by spraying or by using liners on genital surfaces **(33)**.

The production of antimicrobial substances, such as organic acids, hydrogen peroxide and bacteriocins are inhibitory to both Gram positive and Gram negative bacteria **(34, 35)**.In addition that, antimicrobial substances may induce an antagonistic action to pathogens **(36)**.The accumulation of such metabolites such as short chain fatty acids can reduce the pH of the surrounding environment, which can directly suppress the growth of pathogens. Lactic acid bacteria also release other antimicrobial factors as bacteriocins and reuterin **(37, 38)**.

CFS has an antibacterial activity against uropathogens as it contains organic acids that decrease the pH at acidic levels where pathogenic bacteria can't survive **(39, 40)**.These acids may acidify cytoplasm of sensitive microorganisms leading to collapsing electrochemical proton gradient which ultimately causes bacterial death **(41)**. Because of *L. plantarum LPS10* probiotic characters showed previously and its quantitative inhibition of some urogenital pathogens in our work, this organism could be used as a probiotic bacterium **(13)**.

CONCLUSION

Urinogenital infections proved to be one of the most common diseases which mankind can face nowadays. Both men and women can be infected by the urogenital pathogens but the last are considered more susceptible to these injuries due to their physiological nature. The emergence of multi-drug resistance phenomenon made the treatment with such antibiotics is useless. Finding anew remedy was a necessity. Lactobacilli are the dominant bacteria of a healthy human vagina and they exert a significant influence on the microbiology of the vagina. Lactobacilli play a protective role against pathogen colonization by steric exclusion andproduction of inhibitory substances such as lactic acid that reduces the pH of the medium inhibiting the growth of uropathogens. Lactic acid bacteria also produce hydrogen peroxide and bacteriocins that may affect undesirable or pathogenic strains.

Lactobacillus plantarum LPS10 is a probiotic used in our study to control the urogenital pathogenic strains. The CFS of this probiotic could inhibit the growth of *Staphylococcus aureus* after 24h of incubation and the growth of *E. coli* after 48h of incubation. The growth of other pathogens also decreased at different times of incubation. This reflects the ability to use *L. plantarum LPS10* as a probiotic.

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