

Efficiency of Surlactin Produced by *Lactobacillus acidophilus* on Inhibition of Biofilm of *Staphylococcus epidermidis*

Fouad H. K.¹, H.H. Khanaqa², Munira Ch. F.³

Abstract

Objectives: The aims of this study were to screen for the presence of *Lactobacillus acidophilus* in vagina of healthy women and its ability in production and biological activity of its produced surlactin on inhibition of biofilm of *Staphylococcus epidermidis*

Material and Methods: Fifty vaginal swabs were collected from healthy women attending Kamal Al-Samarai and Al-Alweis Maternity Hospitals in Baghdad. Inoculated blood and chocolate agar plates were incubated at 37°C for 18-24 hours. Initial isolations from vaginal samples were done under anaerobic atmosphere. Suspected colonies were first identified by cultural characteristics and Gram's stain. They were further identified by standard biochemical test (API 20A System) subsequently to use of selective media (Man-Rogosa-Sharpe, MRS).

Results: Out of fifty vaginal swabs from healthy women, there were eighteen isolates of *Lactobacillus acidophilus* (36%) were isolated. The *in vitro* ability of these isolates to produced surlactin, in the stationary phase was performed by determining the quantity of protein and carbohydrates by following the standard biochemical procedures. It was found that all isolates of *Lactobacillus acidophilus* isolates produce surlactin but with various extents. The isolates no.15 and no.18 produced highest amount of surlactin which have more efficiency to destroy the biofilm of *Staphylococcus epidermidis* using test tube method. Similar result had been obtained when tested by inhibition of the pathogenic bacteria adhesion to the epithelial cells of urinary tract.

Conclusion: *Lactobacillus acidophilus* was isolated from vaginas of healthy women's, which has ability in production of surlactin but with various extents and possess more efficient activity to destroy the biofilm of *Staphylococcus epidermidis*.

Keywords: *Lactobacillus*, Surlactin, Adherence, biosurfactants.

<http://doi.org/10.33091/AMJ.1400812010>

¹ Erbil Medical Technical Institute.

² Kirkok University

³ Baghdad University , Iraq

Introduction

The urogenital microflora of a healthy woman comprises \approx 50 species of organisms, which differ in composition according to reproductive stages and exposure to several factors, including antibiotics and spermicides ⁽¹⁾. Whilst, the vaginal ecosystem harbors a microbiota that is being increasingly recognized as protecting it from invading pathogens, including those that cause urinary tract infections and sexually transmitted diseases. Lactobacilli are dominant in this habitat, at 107 to 108 CFU/g of vaginal fluid in healthy premenopausal women ⁽²⁾. Among them, those belonging to the *Lactobacillus acidophilus* group and *L. fermentum* are most frequently isolated, although others, such as *L. plantarum*, *L. brevis*, *L. jensenii*, *L. casei*, *L. delbrueckii*, and *L. salivarius*, are isolated as well ⁽³⁾. Lactobacilli are believed to interfere with pathogens by different mechanisms. The first is competitive exclusion of genitourinary pathogens from receptors present on the surface of the genitourinary epithelium ^(4, 5). Second, lactobacilli coaggregate with some uropathogenic bacteria ⁽³⁾, a process that, when linked to the production of antimicrobial compounds, such as lactic acid, hydrogen peroxide, bacteriocin-like substances ^(6, 7), and possibly biosurfactants, it may inhibit the growth of pathogens ⁽⁵⁾. Adherence of bacteria to epithelial cells has been shown to be an important factor in the colonization of mucous membranes. However, little is known about the mechanisms by which lactobacilli from the vaginas of healthy young women adhere to vaginal epithelial cells, although the variety of surface structures in these bacteria implies that a spectrum of adherence mechanisms may exist ⁽²⁾. Furthermore, self-aggregation may substantially increase the colonization potential of lactobacilli in environments with short residence times.

The aims of this study were to screen the presence of *Lactobacillus acidophilus* in vagina of healthy women and its ability in production and biological activity of biosurfactants.

Materials and Methods

Isolation and identification of *Lactobacillus* strain:

50 vaginal swabs were collected from healthy women attending Kamal Al-Samarai and Al-Alweia Maternity Hospitals in Baghdad. Inoculated blood and chocolate agar plates were inoculated incubated at 37°C for 18-24 hours. Initial isolations from vaginal samples were done under anaerobic atmosphere. Suspected colonies were first identified by gram's stain and colony morphologies; they were further identified by standard biochemical test (API 20A System) ^(8, 9), subsequently to use of selective media (MRS).

Biosurfactants production:

For selecting numbers of eighteen *Lactobacillus acidophilus* isolates, 100-ml cultures in MRS broth were grown overnight (for 18 hour) to invest the stationary phase of bacterial growth. The cells were harvested by centrifugation at 6000 rpm/minutes for 30 minutes at 5°C, washed twice in demineralized water, and resuspended in 10 ml of sterile phosphate buffer saline, PBS (pH 7). The lactobacilli were incubated at room temperature for 2 hr. with gentle stirring for biosurfactants production. Subsequently, the bacteria were removed by centrifugation, and the remaining supernatant liquid was filtered through a 0.22-mm-pore-size filter (Millipore). Filtered product was dialyzed against demineralized water at 5°C overnight membrane tube (molecular weight cutoff, 6,000 to 8,000) and subsequently against sucrose to be concentrated.

Detection of biosurfactants:

A- Biosurfactants concentration was determined depended on Dubois method ⁽¹⁰⁾ that are by measuring the concentration of carbohydrate and protein using Bradford method ⁽¹¹⁾. Finally a total of both components could suspect the concentration of glycoprotein.

B-Adherence test:

1-Adherence assays.

Critchley and Douglas method, 1987(12) has been depended on. Urine epithelial cells were collected from healthy women and treated as described previously (13). Overnight cultures of the *S.epidermidis* to be tested were suspended to 10⁴ cells/ml in Tryptone Soya broth (TSB). Equal volumes of the bacterial biosurfactants suspensions were mixed and incubated at 37°C with shaking for 60 min. After that, the suspensions were centrifuged at 3000 rpm/min. for 10 minutes and washed twice with 1 volume of phosphate buffer saline (PBS). The deposit cells were placed on microscope slides, fixed and crystal violet (1%) stained. The assays were started by microscopic investigation (40-x) of epithelial cells, and each determination was performed in duplicate compared with positive control for adherence (epithelial cell with *S.epidermidis* only).

2- Test tube Method:

Overnight cultures of the *S.epidermidis* to be tested were suspended to 10⁴ cells/ml in Tryptone Soya broth (TSB), final volume 5 milliliter. 0.25 milliliter bacterial biosurfactants suspensions were mixed in test tube and incubated at 37°C with shaking for 24-48 hr. Gently discard the test tube which contained and stained with crystal violet stain (1%). Results performed were compared with positive control (*S.epidermidis* growth with out biosurfactants form biofilm on inner surface of the test tube) and negative control (culture media with biosurfactants only).

Results and Discussion

Eighteen isolates of *Lactobacillus acidophilus* (36 %), out of fifty vaginal swabs were obtained from healthy vaginal swabs from Kamal Al-Samarai and Al-Alweia Maternity Hospitals, the other *Lactobacillus* species were twenty two isolates (44%). The rest of isolates belong to other species (*Streptococcus spp.*, *Staphylococcus spp.*, *E.coli*). Our result was consistent with other results which suggested that the *Lactobacillus* make up 50 to 90% of the aerobic vaginal micro flora in women ⁽¹⁴⁾ and also abundant in the aerobic urethral flora of healthy women of reproductive age, accounting for 38% of the aerobic flora ⁽¹⁵⁾.

The affinity of *Lactobacillus* for uroepithelial cells appears to be more important to interfere with the adherence of other pathogens to the vaginal epithelial cells by competitive exclusion. *Lactobacilli* have also been shown to produce antibacterial compounds such as lactic acid, H₂O₂, bacteriocin like substances and possibly Biosurfactants ⁽¹⁶⁾.

For this reason, the prophylactic use of selected *Lactobacillus* strains may be an effective means of restoring the normal microbial flora in the vagina ^(17, 18), thus preventing infections. In recent years, there has been an increasing recognition of the role of *Lactobacilli* in the maintenance of the homeostasis within dynamic ecosystems such as the vagina and in the prevention of colonization and infection caused by pathogenic organisms ⁽¹⁸⁾.

The organisms appear to benefit from biofilm formation by gaining access to nutrients, escaping host immune cells and antimicrobial attack, and having an ability to better control their multiplication. A recurrent biofilm is that various factors, including nutrients, antimicrobials, and arriving bacteria, which change the properties and the composition of the biofilm. In women, other factors such as hormonal concentrations,

particularly estrogen, as well as changes induced by oral contraception, glycogen content, vaginal pH, steroid therapy, immunosuppression, and diseases (e.g., Diabetes mellitus) influence the composition of the bacterial biofilm^(19,20).

The menstrual cycle appears to affect the adherence of lactobacilli to epithelial cells in healthy women in those days corresponding to high circulating concentrations of estrogens which result in a higher adherence in vitro and restored colonization post menopause^(21, 22). Depending on microtiter plate and test tube methods, it was suggested that *S.epidermidus* was more efficient than other microbial isolation from vaginal swaps (*P.aeruginosa*, *E.coli*, and *C.albicans*) in production of biofilm. It is well known that biofilm synthesis controlled genetically, so its inhibition by other microbial does not mine prevention of gene coding (Morris et.al,1999).

Our isolates of *L. acidophilus* are differentiated by their ability to produce surlactin, in addition to their biological activity in inhibition of pathogenic organisms adhesion to the epithelial cells. However the isolates^(15, 18) were more efficient in their activity in quantity of biofilm produced, while the isolate⁽⁵⁾ was the lowest one.

References

- 1- Gregor Reid . Probiotic agents to protect the urogenital tract against infection. American Journal of Clinical Nutrition, Vol. 73, No. 2, 437S-443s.
- 2- Sobel, J. D. Virginities and vaginal flora: controversies abound. Curr. Open. Infect. Dis. 9:42-47.
- 3- Redondo-López, V., R. L. Cook, and J. D. Sobel. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial

- micro flora. Rev. Infect. Dis. 12:856-872.
- 4- Chan, R. C. Y., G. Reid, R. T. Irvin, A. W. Bruce, and J. W. Costerton. Competitive exclusion of uropathogens from human uroepithelial cells by *Lactobacillus* whole cells and cell wall fragments. Infect. Immune. 47:84-89. (1985).
- 5- Velraeds, M. C. M., H. C. van der Mei, G. Reid, and H. J. Busscher. Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. Appl. Environ. Microbiol. 62:1958-1963. (1996).
- 6- McGroarty, J. A., and G. Reid Detection of a *Lactobacillus* substance that inhibits *Escherichia coli*. Can. J. Microbiol. 34:974-978. (1988).
- 7- Reid, G., J. A. McGroarty, R. Angotti, and R. L. Cook, *Lactobacillus* inhibitor production against *Escherichia coli* and co aggregation ability with uropathogens. Can. J. Microbiol. 34:344-351. (1988).
- 8- Collee, J. C.; Fraser, A. G.; Marminon, B. P. and Simmons, A. Laboratory strategy in the diagnosis of infective syndromes. In: Practical medical microbiology. 14th ed. Churchill Livingstone. UK. (1996).
- 9- Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. Infection of the eyes, ear and sinuses. Baily and Scott's Diagnostic Microbiology. 10th ed. Mosby. (1998).
- 10- Dubois, M., Gilles, K. A. Hamilton, J. K., Rebers, P. A. and Amith, F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28, 350-356. (1956).

- 11- Bradford, M. M. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248. (1976).
- 12- Critchley, I.A. and Douglas, L.J. Isolation and characterization of an adhesin from *C. albicans*. *J. Gen. Microbiol.* Vol.133:629-636. (1987).
- 13- Wood, J. R., R. L. Sweet, A. Catena, W. K. Hadley, and M. Robbie. In vitro adherence of *Lactobacillus* species to vaginal epithelial cells. *J. Obstet. Gynecol.* 153:740-743. 1985.
- 14- Redondo-Lopez, V., R. L. Cook, and J. D. Sobel. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial micro-flora. *Rev. Infect. Dis.* 12:856–872. 1990.
- 15- Lin, S. C., M. A. Minton, M. M. Sharma, and G. Georgiou. Structural and immunological characterization of a biosurfactant produced by *Bacilluslicheniformis* JF-2. *Appl. Environ. Microbiol.* 60:31–38. 1994.
- 16- Lawson Reid G., Probiotic agents to protect the urogenital tract against infection. *Am J Clin Nutr*; 73(2 Suppl):437S-443S. (2001).
- 17- Delia A, Morgante G, Rago G *et al.* Effectiveness of oral administration of *Lactobacillus paracasei* subsp. *Paracasei* F19 in association with vaginal suppositories of *Lactobacillus acidophilus* in the treatment of vaginosis and in the prevention of recurrent vaginitis. *Minerva Ginecol* . Jun; 58(3):227-31. (2006).
- 18- Deborah B Nelson, Scarlett Bellamy, Irving Nachamkin, Roberta B Ness, George A Macones, MD, and Lynne Allen-Taylor. First trimester bacterial vaginosis, individual microorganism levels and risk of second trimester pregnancy loss among urban women. *J. Fertil Steril* . November; 88(5): 1396–1403. (2007).
- 19- Soledad Boris,¹ Juan E. Suárez,¹ Fernando Vázquez,² and Covadonga Barbés¹ Adherence of Human Vaginal Lactobacilli to Vaginal Epithelial Cells and Interaction with Uropathogens. *Infect Immun*, May, p. 1985-1989, Vol. 66, No. 5. (1998).
- 20- Galask RP Vaginal colonization by bacteria and yeast. *Am J Obstet Gynecol*; 158:993–5. (1988).
- 21- Chan RCY, Bruce AW, Reid GA Adherence of cervical, vaginal and distal urethral normal microbial flora to human uroepithelial cells and the inhibition of adherence of uropathogens by competitive exclusion. *J Urol*; 131:596–601. (1984).
- 22- Raz R, Stamm WE A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. *N Engl J Med*; 329:753–6. (1993).