Studying the Anti-Adhesion Ability of S-layer Proteins and Filtrate of *Lactobacillus* spp. Against Some Pathogenic Microorganisms *In Vitro*

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**الخلاصة**

أجريت هذه الدراسة لتقديم فعالية البروتينات السطحية المعزولة من بكتريا حامض اللاكتيك العصوي بصورة مقارنة مع فعالية راشح البكتيريا الخام المركز ضد بعض الأحياء المجهرية المرضية داخل الزجاج. استُخدمت (12) عزلة من بكتريا حامض اللاكتيك المعزولة من الخيل و حليب البقر و حليب البقر واللبن و المهبل للكشف عن وجود البروتينات السطحية بـ Polyacrylamide gel electrophoresis (SDS-PAGE) وأستخدم حزمة البروتين السطحي و معاملاتها مع G-HCl (Guanidin hydrochloride) لاسترجاع البروتين من الهمام. قدرت الأوزان الجزيئية للبروتينات وكانت تتراوح ما بين (37- 63 kDa) حسب اختلاف أنواع بكتريا حامض اللاكتيك، كذلك حسب تراكز البروتينات السطحية *Lactobacillus* باستخدام عدد تعتمد في آلية عملها على طريقة البيروتيت. و تم اختيار العزلتين أعتمادا على الوزن الجزيئي و تركيز البروتين. أظهرت نتائج التركيز المثبط الإدائي Lactobacillus casei و *acidophilus* MIC لرواشح مزروع بكتريا حامض اللاكتيك المركز لثلاث مرات ان نسبة 40% و 50% من كلا نوعي بكتري حامض اللاكتيك العصويه ما التراكزين المثبطين الدنيا ضد بكتريا *Escherichia coli* و *Pseudomonas aeruginosa* و أما نسبة 60% فقد كانت التركيز المثبط الإدائي الدنيا ضد *Cadida* و *Salmonella typhimurium* بينما كانت نسبة 50% و 60% نسبة التراكزين المثبطان الدنيا ضد بكتريا *albicans*.

*Staphylococcus aureus* و *E. coli* استخدم التركيز المثبط الإدائي لدراسة ظاهرة الالتصاق لبكترى على الخلايا الطلائيه، و بينت النتائج فعالية الراشح المركز في التقليل من الاصطقاء خلايا هاتين.
Studying the Anti-Adhesion Ability of S-layer Proteins and Filtrate of *Lactobacillus* spp. Against Some Pathogenic Microorganisms *In Vitro*

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ABSTRACT

This project was conducted to evaluate the activity of S-layer proteins extracted from *Lactobacillus* in comparison with the activity of concentrated filtrate of *Lactobacillus* against some pathogenic microorganisms *in vitro*. Twelve isolates of *Lactobacillus* spp. obtained from, vinegar, human milk, cow milk, yoghurt and vagina, were used to detect the S-layer protein (Slp) by Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) then extracted it by excised the Slp pand and treated with 6M guanidin hydrochloride (G-HCl) to eluted the protein from gel. The Molecular weights (MW) of Slps were estimated between (37-63 kDa) depending on the *Lactobacillus* species. The concentrations of Slp were estimated by using a Kit based on the Biuret method. One isolate of each of *Lactobacillus acidophilus* and *Lactobacillus casei*, were selected depending on the MW and concentrations of S-layer proteins. Minimum inhibitory concentrations (MICs) of *Lactobacillus* spp. concentrated filtrates were determined. Results showed 40% and 50% of the concentrated filtrate of both *Lactobacilli* were the MIC for *Pseudomonas aeruginosa*, *Escherichia coli*, respectively, where as MIC for *Salmonella typhimurium* and *Cadida albicans* it was 60%, while 60% and 50% of *L. acidophilus* and *L. casei* respectively, were MIC against *Staphylococcus aureus*. At such MICs, adhesion of *E. coli* and *Staph. aureus* to the uroepithelial cells was minimized when the average decreases recorded were (5-12) and (4-9) bacteria/cell after they were (50-60) and (29-35) bacteria/cell, respectively. Adhesion of *E. coli* and *Staph. aureus* to the uroepithelial cells was also decreased by S-layer proteins with average decreased (3-9) bacteria/cell for both tested bacteria.

INTRODUCTION

The administration of Lactic Acid Bacteria (LAB) contained in fermented foods, especially dairy products, has been found to exhibit a wide range of physiological and therapeutic effects, including enhancement of non-
specific and specific immune responses, suppression of intestinal infection and alleviation of food allergies. However, the protective and immune-enhancing effects of probiotic LAB are known not as genus- or species-specific, but as strains. Accordingly, probiotic LAB strains have become very important in the fields of nutrition, health, and food for research and commercial development. Probiotics LAB have mostly been found in animal sources, dairy products, human and animal intestines (1). An important property proposed for a probiotic bacterium is the ability to adhere and colonize host tissues, which enhances multiplication and survival of bacteria in the host and prevents colonization by pathogenic bacteria. Suppression of the growth of pathogens can also be achieved through competition for nutrients as well as by production of bactericidal components, such as bacteriocins, lactic acid or hydrogen peroxide (2).

*Lactobacilli* interact with the host via several distinct surface components. Adhesion to host tissues is considered to be the first step in bacterial colonization. The role of proteinaceous surface molecules in adhesion has been proposed in several studies (3). Like many other bacteria, several species of *Lactobacillus* have a surface (S-) layer as the outermost component of the cell (4). S-layers are periodic crystalline arrays that are composed of protein or glycoprotein subunits, which form a solid layer to cover the whole cell surface (5). The function of *Lactobacillus* S-layers characterized so far is involved in mediating adhesion to different host tissues. In addition to surface layer proteins (Slps) adhesive properties, the very large number of S-layer subunits present on the cell surface has prompted research aiming at the use of S-layers as a vehicle for the delivery of biologically active compounds, such as drug molecules, antibodies, enzymes and vaccine antigens (6). The members of the genus *Lactobacillus* are important residents of the gastrointestinal (GI) microbiota and have been subjects of increasing interest due to their possible role in the maintenance of GI health. Because of this putative health promoting properties, *Lactobacillus* species are widely used as probiotics. This study aimed to extraction S-layer proteins from *Lactobacillus spp.* of different sources, evaluating the activity of S-layer proteins and *Lactobacillus* concentrated filtrates to inhibit the adhesion of some pathogenic bacteria *in vitro*.
### MATERIALS AND METHODS

#### Bacterial Isolates:
Bacterial isolates used in this study were obtained from different sources as indicated below:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Supplied by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two isolates of <em>Lactobacillus</em></td>
<td>chicken intestine</td>
<td>College of Veterinary Medicine/Baghdad University</td>
</tr>
<tr>
<td><em>Lactobacillus</em> acidophilus</td>
<td>faeces of children</td>
<td>Biotechnology Research Centre/AL-Nahrain University</td>
</tr>
<tr>
<td><em>Lactobacillus</em> casei</td>
<td>faeces of children</td>
<td>Biotechnology Research Centre/AL-Nahrain University</td>
</tr>
<tr>
<td><em>Escherichia</em> coli</td>
<td>Skin infection</td>
<td>Biotechnology Department/College of Science/Al-Nahrain University</td>
</tr>
<tr>
<td><em>Staphylococcus</em> aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> aeruginosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> albicans</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Isolation of *Lactobacillus* from different sources:
Two samples of vinegar, five samples (3ml) of human milk (taken from healthy women), three samples of cow milk, and four of yoghurt were collected in order to isolate *Lactobacillus*, also *Lactobacillus* isolates were isolated from the vagina of healthy premenopausal women by the gynecologist doctor in Kamal AL-Samarai hospital, Baghdad. *Lactobacillus* isolated according to the method was performed by (7).

#### Detection of S-layer proteins:
*Lactobacillus* cells grown in MRS broth were collected by centrifugation at 10,000 rpm for 10 min at 4°C and washed once with 0.5M Tris-HCl, pH 7.5. The pellet, equivalent to 1 ml of culture, was resuspended directly in 200 µl of Laemmli sample buffer and analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis 10% (SDS-PAGE) (8).

#### Extraction of the S-layer protein:
The bands which located in the range between Transferrin and Trypsine was excised and cut into pieces. The protein was eluted from the gel pieces in 1.5 ml of 6 M guanidine hydrochloride-0.5 M Tris-HCl-2 mM EDTA, pH 7.5, by incubating in an end-over mixer at room temperature for 10 h. The eluate was dialyzed against 0.1M Tris-HCl, pH 8.5, at +4°C for 10 h. also analyzed by (SDS-PAGE), In order to ensure the purity of protein. This method was done according to (9).
Determination of Total Protein:
Protein concentration was estimated using specific kit depending on Biuret method.

Determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Lactobacillus spp.* Concentrated Filtrates:
One hundred ml of filtrate of *Lactobacillus* was concentrated by oven at 40-45 °C to one- fold (50 ml), two -fold (25 ml) and three- fold (12.5 ml).
Serial dilutions (10ml) of three fold concentrated filtrate of *Lactobacillus* were made in tubes containing sterile nutrient broth. The ratios were (10, 20, 30, 40, 50, 60, 70, 80 and 90%) giving final volume of 10 ml in each tube. After each concentration was inoculated by 0.1 ml of the test organisms (*P. aeruginosa, E. coli, Staph. aureus, Sal. typhimurium* and *C. albicans*), it was incubated at 37 °C for 24 hr. Growth intensity of each tube was observed by inoculation on nutrient agar and Sabouraud dextrose agar (for *Candida albicans*) then incubated overnight at (37°C) Results were recorded as growth (+), and no growth (-) (10).

Bacterial Adhesion Test (11):-
Preparation of *E. coli* and *Staph. aureus*:
Ten milliliter of nutrient broth medium was inoculated with bacterial growth culture, and incubated at 37°C for 24 hr. After that, the culture of bacteria was collected by centrifugation at 1000 rpm for 20 min then, washed twice with PBS and concentrated by centrifugation at 1000 rpm for 20 min and resuspended in PBS.

Preparation of Epithelial Cells:
Uroepithelial cells were isolated from urine of some healthy females by centrifugation at 1000 rpm for 5 min then washed three times with 5ml of PBS and recentrifuged at 1000 rpm for 10 min before resuspension in 5ml of PBS.

Adhesion Test:
- A mixture of bacterial suspension, and epithelial cells suspension (0.2 ml for each) beside 0.1 ml of PBS were incubated at 37°C for 1 hr.
- Unattached bacterial cells to uroepithelial cells were removed by centrifugation in 5ml of PBS at 1000 rpm for 10 min. The filtrate was ignored.
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- The pellet was resuspended in PBS. A drop of suspension was transferred to a microscopic slide, air-dried, fixed with methanol: acetic acid (3:1) and stained with methylene blue.
- The adhered bacterial cells to epithelial cells were observed by the compound light microscope.
- The control contained only epithelial cells.

Effect of Concentrated Filtrates on Adhesion Property of Tested Organisms:

The minimum inhibitory concentration of the concentrated filtrates of Lactobacillus spp. isolate was used to investigate its effect on adhesion property of tested organisms on uroepithelial cells in vitro as following:

Nutrient broth medium containing minimum inhibitory effect of concentrated filtrates was dispensed in sterile tubes and incubated with a loopful of each liquid culture of the tested bacteria at (37°C) for (24) hr.

Adhesion test as mentioned above was reused to examine inhibitory effect of the concentrated filtrate after treatment.

Effect of S-layer proteins on Adhesion of Tested Organisms (12):

Mixtures consisted of 0.2 ml from each of the following, bacterial suspension, epithelial cells suspension and S-layer proteins isolated from Lactobacillus spp. were incubated at 37°C for 1 hr. Procedure was completed as mentioned above.

RESULTS AND DISCUSSION

S-layer proteins and their extraction with Guanidine HCl:

Presence of crystalline arrays of protein (that so-called S-layer) covering the cell surface has been shown in several Lactobacillus species (13).

Putative S-layer proteins on the bacterial cell surface can be deduced by the occurrence of a dominant protein band in the protein profile of non-lysed bacteria.

Twelve isolates of Lactobacillus spp. were analyzed by electrophoresis using 10% SDS-PAGE and the lane of proteins bands obtained were compared with four marker proteins (γ-globulin MW = 150 kDa, Transferrin MW = 80 kDa, Trypsine MW = 20 kDa, Lysozyme MW = 14 kDa).
To extract S-layer protein, the band which located between Transferrin and Trypsine excised and treated with 6M G-HCl from crude column.

(4) Found that *Lactobacilli* surface layer proteins are among the smallest detected with molecular masses ranging from 25 to 71 kDa. The S-layer subunits are non-covalently linked to each other and to the supporting cell envelope, and can be disintegrated into monomers by denaturing agents such as urea or guanidine HCl, metal-chelating agents or by cation substitution (14).

Results of protein profile by SDS-PAGE revealed that seven bands with MW range between 10.26-108.71 kDa were obtained after analysis of *L. acidophilus* isolate (1) which isolated from chicken intestine. Then, detected band were excised and treated with 6M guanidine hydrochloride and analysed by SDS-PAGE. Results showed that only one band was obtained with MW 47.74 KDa. It was corresponded to the original band in crud column as shown in figure (1). This came in accordance to (15) who mentioned that S-layer proteins of *lactobacilli* have molecular mass between 40 and 55 kDa.

Analysis of protein profile of *L. acidophilus* (2) isolate from chicken intestine gave eight bands with MW range between 11.50-177.74 kDa. Band with MW 50 kDa represented the S-layer protein, and treatment of this band with 6M guanidine hydrochloride gave one band with MW 48.37 kDa which corresponded to the original band in crud column (figure, 1).

![Fig. 1: Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (A) L. acidophilus1 and (B) L. acidophilus2 isolated from chicken intestine.](image)

L1: Protein markers, L2: Crude analysis and L3: Pure S-protein analysis

Results of protein profile analysis of *L. acidophilus* from feces of children showed seven bands with MW ranged between 13.10 -147.53 kDa. Band with MW 49.46 KDa represented the S-layer protein. On the other hand, five bands were obtained from *L.casei* of children feces with MW range between 14.37- 292.50 KDa. Only band with MW 43.59 KDa represented S-layer protein.
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Analysis S-layer from *L. acidophilus* and *L. casei* after treating with 6M guanidine hydrochloride gave two bands with MW 49.46 and 44.66 KDa, respectively as shown in figure (2).

![Fig. -2 : Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (A) *L. acidophilus* (B) *L. casei* isolated from faeces of children, L1: Protein markers, L2: Crude analysis and L3: Pure S-protein analysis](image)

Results in figure (3) indicated that bands with MW 46.52 and 44.25 KDa represented the S-layer protein of *L. plantarum* and *L. acidophilus* isolated from yoghurt, respectively. Treatment of these bands with 6M guanidine hydrochloride gave bands with MW 48.69 and 43.42 kDa, respectively, which were corresponded to the original band in crud column. (16) Found that the molecular weight of surface protein was 43 kDa when extracted from *L. acidophilus* ATCC 4356 by treatment of whole cells with 4 M guanidine hydrochloride.

Analysis of protein profile of *L. gasseri* from human milk gave seven bands with MW range between 13.70 – 158.94 KDa. Band with 38.92 kDa represented S-protein; treatment of this band with 6M guanidine hydrochloride gave one band with MW 37.58 KDa was corresponded to the original band in crud column as shown in figure (4).
Fig. -3 : Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (A) *L. plantarum* (B)*L.acidophilus* isolated from yoghurt

Fig. -4 : Protein profile analysis of *Lactobacillus gasseri* isolated from human milk by 10% SDS-PAGE  
L1: Protein markers, L2: Crude analysis and L3: Pure S-protein analysis

S-layer proteins did not appeared in protein profile analysis of *L. fermentum* isolated from vagina (figure, 5). This result was disagreed with that of (17) who purified and characterized a 29-kDa cell surface protein from *L. fermentum*.

(18) Stated that among lactic acid bacteria, the S-layer seems to be a typical surface structure in several *Lactobacillus* species, e.g., in *L.acidophilus, L. helveticus, L. casei, L. brevis, L. buchneri, L.fermentum, L. bulgaricus*, and *L. plantarum*. 
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**Fig. -5:** Protein profile analysis of *Lactobacillus fermentum* isolated from vagina by 10% SDS-PAGE.
L1: Protein markers, L2: Crude analysis and L3: Pure S-protein analysis

Results of analysis of protein profile of *Lactobacillus plantarum* from vinegar, showed that only one band with MW of 63.06 kDa was visible, while *Lactobacillus plantarum* from cow milk gave S-layer band with MW 51.46 kDa, as indicated in figure (6).

**Fig. -6:** Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE (A) *L. plantarum* from vinegar (B) *L. plantarum* from cow milk.
L1: Protein markers, L2: Crude analysis and L3: Pure S-protein analysis

Protein profile analysis of *L. rhamenosus* and *L. curvatus* from cow milk showed that two bands were obtained with MWs 60.09 and 39.64 kDa, respectively, (figure, 7).
(19) Found that the molecular masses of S-layer proteins of *Lactobacillus spp.* which isolated from pig intestine ranging between 45–62 kDa.

The molecular weight of S-layer protein is varied depending on species and sources of *Lactobacillus*. Most S-layers are composed of a single protein species which greatly varies in size related to different bacterial genera (20).

![Fig. -7](image)

**Fig. -7**: protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (K) *L. rhamenosus* (L) *L. curvatus* isolated from cow milk.

L1: represents protein markers, L2: represents crude analysed cells of *Lactobacillus* and L3: is pure protein.

**Concentrations of *Lactobacillus* S-layer proteins:**

The concentrations of extracted S-proteins from *Lactobacillus* were determined by using Kit which depended on Biuret method.

Results of the concentrations of S-proteins showed that were ranged from 1.87 mg/ml for *L. acidophilus* (isolated from chicken intestine) to 0.13 mg/ml for *L. curvatus* (from cow milk) as shown in table (1). Under laboratory cultivation conditions, yield of the S-layer glycoprotein ranges between 0.5 and 2.0 g wet weight per litre of growth medium (21).

**Table -1**: Concentrations of S-layer proteins of *Lactobacillus* isolates

<table>
<thead>
<tr>
<th>S-layer protein from</th>
<th>Concentration of protein (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolates</strong></td>
<td><strong>sources</strong></td>
</tr>
<tr>
<td><em>L. acidophilus1</em></td>
<td>From chicken intestine</td>
</tr>
<tr>
<td><em>L. acidophilus2</em></td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>From feces</td>
</tr>
<tr>
<td><em>L. casei</em></td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>From yoghurt</td>
</tr>
</tbody>
</table>
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| **L. acidophilus** | 0.32 |
| **L. gasseri** | From human milk | 0.55 |
| **L. plantarum** | From vinegar | 1.21 |
| **L. plantarum** | From cow milk | 0.57 |
| **L. rhamenosus** | 1.17 |
| **L. curvatus** | 0.13 |

Two S-layer proteins extracted from *L. acidophilus* and *L. casei* which their molecular weight were (47 and 44 kDa) and their concentrations were (1.87 and 1.39 mg/ml), respectively, were used in this study to evaluate the biological role of S-layer proteins.

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of LAB Filtrates against pathogens:**

Results (table 2) indicate that concentrations 10% and 20% of both *L.a* and *L.c* filtrates had no effect on the tested microorganism when clear growth of pathogenic microorganisms was observed after (24hr) of incubation. Adversely, 40% of both filtrates led to minimized growth (MIC) of *P. aeruginosa*, while concentration 50% of both LAB filtrates was needed to inhibit growth of this bacterium completely (MBC).

At the time that a sharp decrease in growth of *E. coli* was recorded by treatment with concentration 50% of both LAB filtrates, growth was completely inhibited by 60% concentration. The concentrations of 50 % and 60 % of *L. acidophilus* and *L. casei* filtrates respectively, were considered the MICs against *Staph.aureus*, where as 60 % and 70 % were the MBCs. Concentration 60% of both *L.a* and *L.c* filtrates (MIC) were sharply reduced growth of *Sal. typhimurium* and *C. albicans*, while 70 % completely inhibited their growth. (22) found that concentration 50% of LAB was the MIC for *E. coli*, while 60% for *Staph.aureus* and *P. aeruginosa*. (23) found that the MIC of *L.acidophilus* and *L. plantarum* concentrated filtrates were 50% and 60% respectively, for *Proteus mirabilis* isolates.
Table -2: Minimum Inhibitory Concentrations (MIC,s) and Minimum Bactericidal Concentrations (MBC,s) of Concentrated Filtrates of *L. acidophilus* and *L. casei* against pathogens:

<table>
<thead>
<tr>
<th>Isolates</th>
<th>LAB filtrate concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Cadida albicans</em></td>
<td>+</td>
</tr>
</tbody>
</table>

Growth = +   No Growth= -

**Adhesion of *Escherichia coli* and *Staphylococcus aureus*:**

Adherence of pathogenic bacteria to host epithelial cells is an important step in the initiation of the infectious process (24). Bacterial adhesion is initially based on non-specific physical interaction between two surfaces, which then enable specific interaction between adhesion usually (proteins) and complementary receptors (25). In the current study, adherence property of *E. coli* and *Staph. aureus* as well as how this property may be affected by LAB isolate and S-proteins, was investigated.

Adherence ability of *E.coli* and *Staph. aureus* to uroepithelium (UEP) is shown in (Figure 8). Results clarified that the average number of *E. coli* adhering to UEP ranged from 50-60 bacteria/cell, whereas the number of *Staph. aureus* adhering to UEP ranged from 29-35 bacteria/cell.

Many researches confirmed that pili mediate attachment of uropathogenic *E. coli* to human urinary tract epithelium (26) while *Staph. aureus* and *streptococci pyogenes* adhere to host epithelial cells through the expression of surface proteins which bind to the host extracellular matrix proteins such as fibronectin and collagen (27).
Fig. -8: Microscopical Examination of Adhesion Property of E. coli and S. aureus Uroepithelium cell under Oil - Immersion Objective (100xs). (A) Normal Uroepithelial cell (B) E.coli Adhered to Uroepithelial Cell (C) S. aureus adhered to Uroepithelial Cell.

**Adhesion Inhibition by LAB Filtrates and S-layer protein:**

The effect of concentrated filtrate of LAB and S-layer protein against adhesion property of E. coli and Staph. aureus were studied. Results showed that the three-fold concentrated filtrate of LAB (L.acidophilus and L.casei) minimized adhesion of E. coli to uroepithelial cell reaching an average of (5-12) bacteria / cells (fig. 9.A). In this aspect (28) found that L. fermentum produced a proteinaceous component detectable in spent culture fluid during growth in both complex and defined media; this component inhibited the adhesion of E. coli fimbriae to ileal mucus by interacting with mucus components.

The three-fold concentrated filtrates of both LAB minimized adhesion of Staph. aureus to the uroepithelial cell reached an average of (4-9) bacteria / cells (fig. 3-9.B). Study of (29) found that precoating of LAB strains reduced the binding of uropathogenic (Staphylococci and E. coli) to 8 bacteria /cell.

Similar reduction also observed when the S-layer protein was used and, adhesion E. coli and Staph. aureus to the uroepithelial cells reached an average of (3-9) bacteria / cells. S-layer protein has the potential to play a role in the competitive exclusion of pathogens (5). (30) who found that S-layer protein extracted from L. helveticus had inhibition effect on enterohaemorrhagic E. coli adhesion to host epithelial cells.
Fig. -9: Microscopical Examination of Adherence of *E.coli* and *Staph. aureus* to the Uroepithelium Cells after Treatment with the Concentrated Filtrate of LAB and S-layer protein (100 X).

-A- After treating *E. coli* with three-fold of LAB.

-B- After treating *Staph. aureus* with three-fold of LAB.

-C- After treating *E. coli* with Slp.

-D- After treating *Staph. aureus* with Slp.

Three-fold concentrated filtrates of LAB and S-layer protein had effect on the adhesion of *E.coli* and *Staph. aureus*.

**REFERENCES**


