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# Evaluation Activity 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 isolated from *Lactobacillus* in comparison with the activity of concentrated filtrate of *Lactobacillus* against some pathogenic microorganisms and against tumor cell lines *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 guanidine hydrochloride (G-HCl) to elute the protein from the 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.

The inhibitory effect of *Lb. acidophilus* and *Lb. casei* was determined against pathogenic microorganism; *Pseudomonas aeruginosa* , *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Candida albicans* on solid and liquid MRS media. Results revealed that less inhibitory activity against them was detected on the solid medium, compared to the liquid one. Greatest inhibitory effect of *Lb. acidophilus* and *Lb. casei* has appeared against *P. aeruginosa* when the zone of inhibition reached to 24 and 22 mm, respectively. It appeared that the inhibitory effect of *Lb. acidophilus* was more than that of *Lb. casei* against most of the tested microorganisms, while S-layer proteins have no effect against pathogenic microorganisms.

## INTRODUCTION

Probiotics lactic acid bacteria (LAB) have been expected to become a useful tool that could be used as a preventive substance instead of antibiotics. However, heavy use of antibiotic has become a major problem, since it results in drug-resistant bacteria, thus, alternative and non-pharmaceutical strategies for controlling enteropathogenic bacterial infection has been sought. Infection by oral challenge with such enteropathogens as

*Salmonella typhimurium*, *Escherichia coli*, *Shigella sonnei* and *Listeria monocytogenes* has been efficiently suppressed by probiotic LAB feeding to rodents (Michail and Abernathy, 2002). 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 the production of bactericidal components, such as bacteriocins, lactic acid or hydrogen peroxide (Reid and Burton, 2002).

*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 (Lorca *et al.*, 2002). Like many other bacteria, several species of *Lactobacillus* have a surface (S-) layer as the outermost component of the cell (Åvall- Jääskeläinen and Palva, 2005). 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 (Sára and Sleytr, 2000). 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 (Sleytr *et al.*, 2007). This study aimed to extraction S-layer proteins from *Lactobacillus spp.* of different sources, evaluating the antimicrobial activity of S-layer proteins in comparison with the concentrated filtrate of *Lactobacillus in vitro*.

## MATERIALS AND METHODS

**Bacterial Isolates:**

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

| Isolate | Source | Supplied by |
|---|---|---|
| Two isolates of *Lactobacillus acidophilus* | chicken intestine | College of Veterinary Medicine/ Baghdad University |
| *Lactobacillus acidophilus* | faeces of children | Biotechnology Research Centre /AL-Nahrain University |
| *Lactobacillus casei* | | |
| *Escherichia coli* | Injury infection | Biotechnology Department/College of Science/Al-Nahrain University |
| *Staphylococcus aureus* | | |
| *Pseudomonas aeruginosa* | | |
| *Salmonella typhimurium* | | |
| *Candida albicans* | | |

**Isolation of *Lactobacillus* from Different Sources**:

Two samples of vinegar, five (3ml) of human milk (taken from healthy women), three 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 Buck and Gilliland in 1995.

**Detection of S-layer Proteins:**

*Lactobacillus* cells grown in MRS broth were collected by centrifugation at 10,000 rpm for 10 min at 4ºC and washed once with 0.5M Tris-HCl, pH 7.5. The pellet, equivalent to 1 ml of culture, was dissolved directly in 200 µl of Laemmli sample buffer and analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis 10% (SDS-PAGE) (Laemmli, 1970).

**Extraction of the S-layer Protein:** (Vidgren *et al*., 1992)

The bands which located in the range between Transferrin and Trypsin 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.

**Determination of Total Protein:**

Protein concentration was estimated by using the specific kit which depended on Biuret method.

**Testing the Inhibitory Activity of Lactic Acid Bacteria (LAB):-**

**On Solid Medium (MRS Agar):**

A culture of *Lactobacillus* was inoculated in MRS broth then incubated anaerobically at 37 °C for 24 hr., then the culture was streaked on MRS agar plates and incubated at 37 ºC for 24, 48 and 72 hr.

After incubation, discs were made from the cultured agar using sterile cork borer (5mm). The discs were fixed on the surface of nutrient agar plates that were previously inoculated with the test bacteria (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*, and *Salmonella typhimurium*) and Sabouraud dextrose agar plate that was previously inoculated with *Candida albicans*, then incubated at 37 ºC for 24 hr. Inhibition zones around the discs were measured by millimeter (Silva *et al*., 1987).

**In Liquid Medium (MRS Broth):**

The tube containing MRS broth medium was inoculated with 1% of a fresh culture of each *Lactobacillus* isolates, then incubated anaerobically at 37 ºC for 24, 48 and 72 hr. After incubation, the culture was centrifuged at 6000 rpm for 15 min., the supernatant was taken and sterilized by filtration (Erdo rul and Erbilir, 2006). Inhibitory effect of *Lactobacillus* crude filtrate was examined against test organisms (*P. aeruginosa*, *E. coli*, *Sapht. aureus*, *Sal. typhimurium* and *C. albicans* ) using well diffusion method (Ryan *et al*., 1996).

One hundred ml of filtrate was concentrated by the oven at 40-45 °C to one-fold (50 ml), two -fold (25 ml) and three-fold (12.5 ml). Well diffusion method was used to detect the effect of each concentrated filtrate against test organisms (*P. aeruginosa*, *E. coli*, *Staph. aureus*, *Sal. typhimurium* and *C. albicans*). The control containing concentrated MRS broth without *Lactobacillus spp.* was used.

**Testing the Inhibitory Activity of S-Layer Proteins:**

To examine the inhibitory effect of S-Layer protein isolated from *Lactobacillus*, well diffusion method was used but using S-layer protein instead of *Lactobacillus* crude filtrate.

## 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 (Boot *et al.*, 1996).

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 was 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 Trypsin excised and treated with 6M G-HCl from the crude column. Åvall-Jääskeläinen and Palva (2005) 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 (Sara, 2001).

Results of protein profile by SDS-PAGE revealed that seven bands with MW range between 10-108 kDa were obtained after analysis of *L. acidophilus* isolate (1) which isolated from the chicken intestine. Then, the 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 KDa. It corresponded to the original band in crud column as shown in figure (1). This came in accordance to Frece, *et al*. (2005) who mentioned that S-layer proteins of *lactobacilli* have a 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 11-177 kDa. The 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 kDa which corresponded to the original band in crud column (figure, 1).

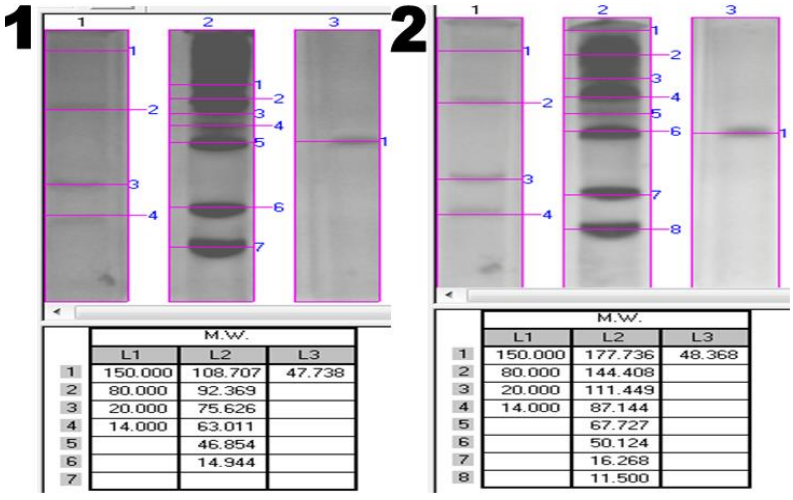



**Fig. (1):** Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (1) *L. acidophilus*1 and (2) *L. acidophilus*2 isolated from the chicken intestine.

Results of protein profile analysis of *L. acidophilus* from feces of children showed seven bands with MW ranged between 13 - 147 kDa. The band with MW 49 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- 292 KDa. The only band with MW 43 KDa represented S-layer protein.

Analysis S-layer from *L. acidophilus* and *L. casei* after treating with 6M guanidine hydrochloride gave two bands with MW 49 and 44 KDa, respectively as shown in figure (2).

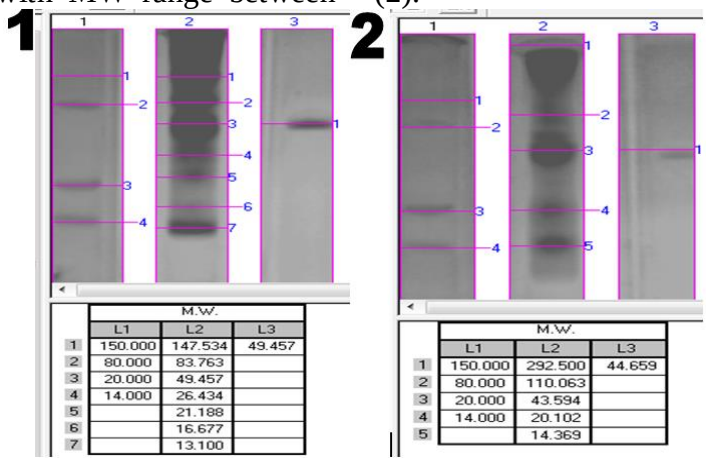



**Fig. (2):** Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (1) *L. acidophilus* (2) *L. casei* isolated from faeces of children

Results in figure (3) indicated that bands with MW 46 and 44 KDa represented the S-layer protein of *L. plantarum* and *L. acidophilus* isolated from yoghourt, respectively. Treatment of these bands with 6M guanidine hydrochloride gave bands with MW 48 and 43 kDa, respectively, which were corresponded to the original band in crud column. Boot *et al* (1993) 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 - 158 KDa. The band with 38 kDa represented S-protein; treatment of this band with 6M guanidine hydrochloride gave one band with MW 37 KDa was corresponded to the original band in crud column as shown in figure (4).

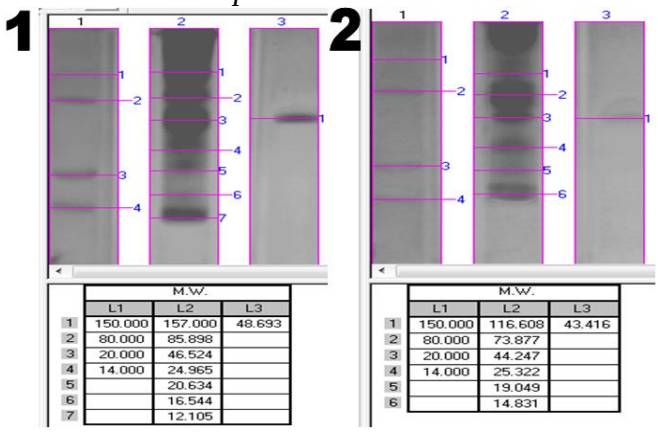
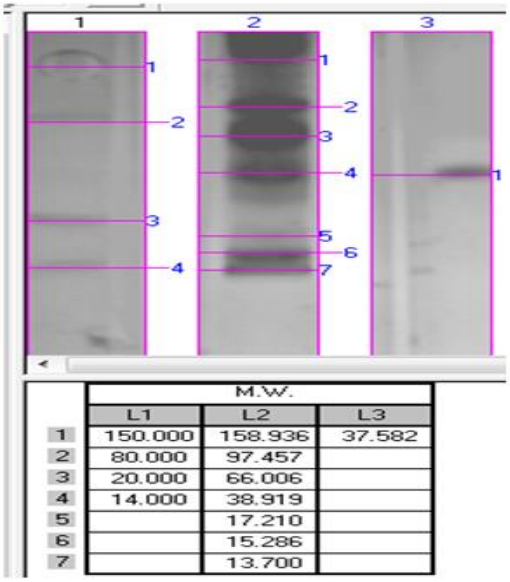



**Fig (3):** Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (1) *L. plantarum* (2)*L.acidophilus* isolated from yoghurt

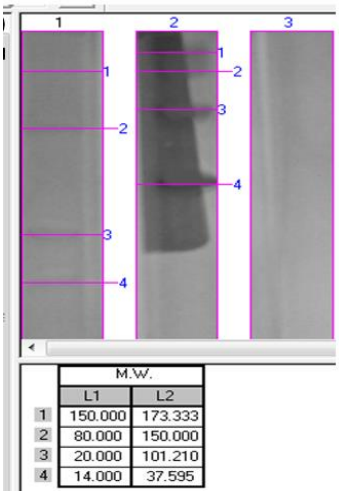



| | L1 | L2 | L3 |
|---|---|---|---|
| 1 | 150.000 | 158.936 | 37.582 |
| 2 | 80.000 | 97.457 | |
| 3 | 20.000 | 66.006 | |
| 4 | 14.000 | 38.919 | |
| 5 | | 17.210 | |
| 6 | | 15.286 | |
| 7 | | 13.700 | |

**Fig. (4):** Protein profile analysis of *Lactobacillus gasseri* isolated from human milk by 10% SDS-PAGE

S-layer proteins did not appeare in protein profile analysis of *L. fermentum* isolated from the vagina (figure, 5). This result has disagreed with that of **Rojas** *et al* (2002) who purified and characterized a 29-kDa cell surface protein from *L. fermentum*.

Kahala, *et al*. (1997) 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* .




| | L1 | L2 |
|---|---|---|
| 1 | 150.000 | 173.333 |
| 2 | 80.000 | 150.000 |
| 3 | 20.000 | 101.210 |
| 4 | 14.000 | 37.595 |

**Fig. (5):** Protein profile analysis of *Lactobacillus fermentum* isolated from the vagina by 10% SDS-PAGE**.**

Results of analysis of protein profile of *Lactobacillus plantarum* from vinegar, showed that only one band with MW of 63 kDa was visible, while *Lactobacillus plantarum* from cow milk gave S-layer band with MW 51 kDa, as indicated in figure (6).

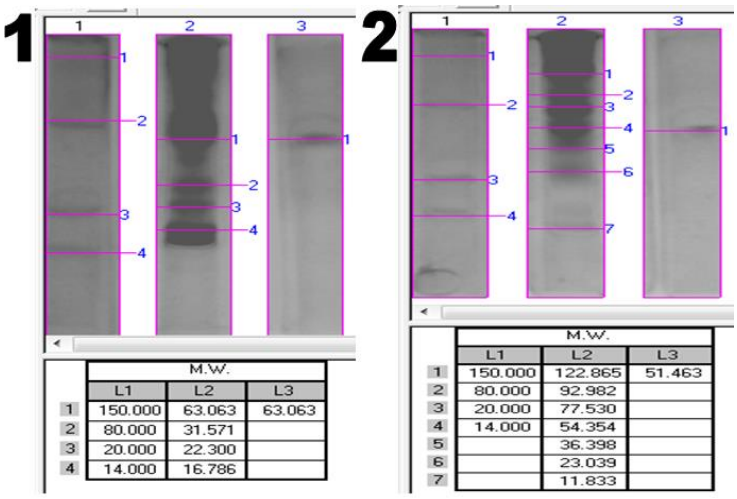



**Fig. (6):** Protein profile analysis of *Lactobacillus* by 10% SDS-PAGE (1) *L.plantarum* from vinegar (2) *L.plantarum* from cow milk.

Protein profile analysis of *L. rhamenosus* and *L. curvatus* from cow milk showed that two bands were obtained with MWs 60 and 39 kDa, respectively, (figure, 3-7).

Jakava-Viljanen and Palva (2007) 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 (Boot and Pouwels, 1996).

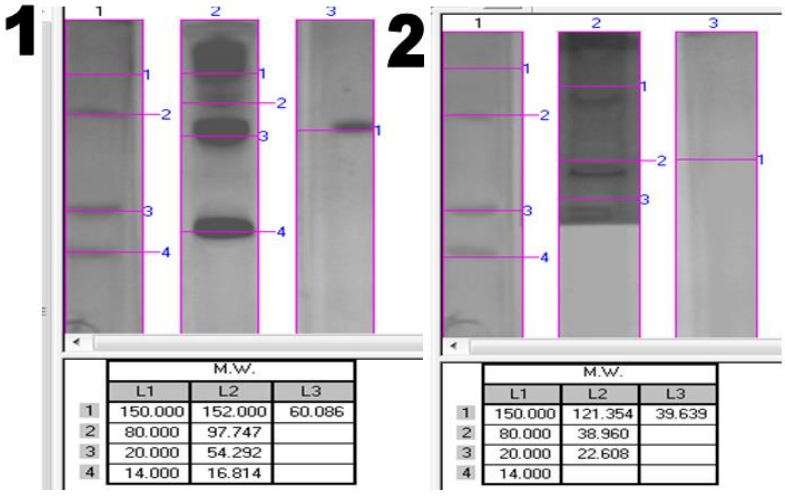



**Fig. (7):** protein profile analysis of *Lactobacillus* by 10% SDS-PAGE: (1) *L. rhamenosus* (2) *L. curvatus* isolated from cow milk. Photocapt analysis software was used to determine the molecular weight of proteins and tables designed by the program in which the molecular weight is calculated in kDa.

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

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

Results of the concentrations of S-proteins showed that were ranged from 1.87 mg/ml for *L.acidophilus* (isolated from the chicken intestine) to 0.13 mg/ml for *L. curvatus* (from cow milk) as shown in the table (1). Under laboratory cultivation conditions, the yield of the S-layer glycoprotein ranges between 0.5 and 2.0 g wet weight per liter of growth medium (Eshinimaev *et al*., 2002).

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

| S-layer protein from | | Concentration of protein (mg/ml) |
|---|---|---|
| **Isolates** | **sources** | |
| ***L. acidophilus1*** | From chicken intestine | 1.87 |
| ***L. acidophilus2*** | | 1.79 |
| ***L. acidophilus*** | From feces | 1.56 |
| ***L. casei*** | | 1.39 |
| ***L. plantarum*** | From yoghurt | 0.83 |
| ***L. acidophilus*** | | 0.32 |
| ***L. gasseri*** | From human milk | 0.55 |
| ***L. plntarum*** | From vinegar | 1.21 |
| ***L. plantarum*** | From cow milk | 0.57 |
| ***L. rhamenosus*** | | 1.17 |
| ***L. curvatus*** | | 0.13 |

In this study, S-layer proteins of *L. acidophilus*1 and *L.casei* were used depending on their molecular weight (47 and 44 kDa) and their concentrations (1.87 and 1.39 mg/ml) respectively.

**Inhibitory activity of *Lb. acidophilus* and *Lb. casei* on Pathogenic Microorganisms:**

**On Solid Medium (MRS agar):**

The ability of *L. acidophilus* and *L.casei* to produce inhibition activity against pathogenic organism was tested by growing the isolates on MRS agar medium for different incubation periods (24, 48, 72 hr) at 37 ºC under anaerobic conditions. In this approach, Anas *et al* (2008) found that several strains of *Lactobacillus* gave inhibition zones against *Staphylococcus aureus* on solid medium ranging from 3-8mm.

Table (2) shows the inhibitory activity of *L. acidophilus* and *L. casei* has grown on MRS agar against the test organisms at three different incubation periods. Composition of MRS media induce LAB to produce secondary metabolites with inhibitory effect against pathogenic bacteria and as it was mentioned by Gauer and Mariana (1994) who found that the production of inhibitory materials by LAB is depended on the media used for growth and they found that Tween 80 induce the production of proteins (bacteriocine) by increasing the activity of the bacteria,. Highest effect was against *P. aeruginosa* after incubation for 24 hr. which reached 14 and 11 mm for *L. acidophilus* and *L. casei*, respectively, this result agreed with Mobarez *et al* (2008) who mentioned that antibacterial activities of bacteriocins from *L. acidophilus* against *P. aeruginosa* was stronger than against *S. aureus*.

**Table (2):** Inhibitory effect of *Lb. acidophilus* and *Lb. casei* against the test pathogenic microorganisms on solid media estimated by the diameter of inhibition zone (mm).

| Microorganism | Incubation time (hr) | Inhibition Zone Diameter of (mm) | |
|---|---|---|---|
| | | *Lb.acidophilus* | *Lb.casei* |
| ***Pseudomonas aeruginosa*** | **24** | **14** | **11** |
| | **48** | **10** | **8** |
| | **72** | **7** | **5** |
| ***Escherichia coli*** | **24** | **3** | **5** |
| | **48** | **9** | **11** |
| | **72** | **5** | **8** |
| ***Salmonella typhimurium*** | **24** | **10** | **9** |
| | **48** | **7** | **5** |
| | **72** | **4** | **-** |
| ***Staphylococcus aureus*** | **24** | **9.5** | **9** |
| | **48** | **8** | **8** |
| | **72** | **4** | **2** |
| ***Candida albicans*** | **24** | **9** | **8** |
| | **48** | **7** | **5** |
| | **72** | **4** | **-** |

The incubation period of 24 hr. gave the highest inhibitory effect by *L.acidophilus* and *L.casei* against *P. aeruginosa*, *Staph. aureus*, *Sal. typhimurium* and *C. albicans* than other incubation periods of 48 and 72hrs, these results came in accordance with those obtained by AL-Marsoomy (2008) who found that *L. plantarum* and *L.casei* gave best inhibitory effect against *E. coli* after 24hr, while disagreed with Al-Dulemy (2000) who found that the inhibitory effect of LAB increased after 48 hr. of incubation, but the inhibitory effect of this *Lactobacillus* against *E. coli* was better after 48 hr. of incubation this result agreed with AL-Yas (2006) who reported that the inhibitory effect of LAB against *H. pylori* increased after 48 hr. and that means the *E. coli* was more susceptible to compounds produced from LAB after 48 hr. when compared with other tested microorganism. Barfoot and Klaenhammer (1983) declared that the death of tested bacteria increased with increasing inhibitory substances like bacteriocin, acidophilin and plantaracin of LAB.

Differences in the above results of LAB against the pathogenic bacteria may be related to the type of bacteria, type of the inhibitory substance, its quantity and ability to distribute in the medium (Egorov, 1985). Aktypis *et al*. (1998) suggested that such differences in the inhibitory effect at different incubation periods may be related to the nature of LAB isolates used against the test bacteria itself.

The present result confirmed that *L.acidophilus* showed a better inhibitory effect against *P. aeruginosa*,*Staph. aureus*, *Sal. typhimurium* and *C. albicans* than *L. casei*. Other studies also clarified the inhibitory effect of *L. acidophilus* as the study of AL-Jeboury (2005) who found that the inhibitory effect of *L. acidophilus* was high against *Proteus mirabilis*, as well as Coollborn (2005) who recorded that *L.acidophilus* exhibit greatest inhibitory effect than other *Lactobacillus spp.* against

the pathogenic organism. *L. casei* gave more inhibitory effect than *L.acidophilus* against *E. coli* only after 48 hr.. This result came in accordance with that obtained by Pishva *et al* (2009) who found that *L. casei* possessed more inhibitory effect against *E. coli* than other *Lactobacillus spp.*

**In Liquid Media:**

Table (3) showed the inhibitory effect of *L. acidophilus* and *L. casei* were more effective against *P. aeruginosa* when inhibition zone reached to 24 and 22 mm in diameter respectively. Maximum inhibition zone diameters reached 24 mm for *L.acidophilus* which was higher than that recorded against same microorganisms on solid medium (14mm). This may be due to MRS broth stimulate more inhibitory compound than MRS agar. This result agreed with Gupta *et al.* (1998) Who mentioned that MRS broth stimulated inhibitory effect against Gram positive (*Staph. aureus, Baccillus subtalis*) and Gram negative bacteria ( *E.coli, klebsiella spp., Proteus spp.)*

Results in the table (3) revealed that *L.acidophilus* gave inhibitory effect against *P. aeruginosa, Staph. aureus, Sal. typhimurium* and *C. albicans* better than that gave by *L. casei* this may due to acidophilin production from *L.acidophilius* as Lewus *et al*. (1991) reporteded. *L. casei* gave more inhibitory effect than *L.acidophilus* against *E. coli*, when inhibitory zone reached 15 mm after 24 hr. of incubation time, while *L.a* has 12 mm after the same of incubation time, this result similar with those obtained on solid medium.

**Table(3):**Inhibitory Effect of *L.acidophilus* and *L.casei against* some pathogenic microorganisms in liquid media estimated by the diameter of inhibition zone (mm).

| Microorganism | Incubation time (hr) | Inhibition Zone of Diameter(mm) | |
|---|---|---|---|
| | | ***L.acidophilus*** | ***L.casei*** |
| *P. aeruginosa* | 24 | 24 | 22 |
| | 48 | 21 | 20 |
| | 72 | 17 | 15 |
| *E.coli* | 24 | 12 | 15 |
| | 48 | 18 | 19 |
| | 72 | 15 | 16 |
| *Sal. typhimurium* | 24 | 17 | 16 |
| | 48 | 15 | 14 |
| | 72 | 13 | 11 |
| *Staph.aureus* | 24 | 17 | 14 |
| | 48 | 15 | 13 |
| | 72 | 12 | 10 |
| *C. albicans* | 24 | 18 | 17 |
| | 48 | 15 | 14 |
| | 72 | 13 | 11 |

The results of the inhibitory effect for concentrated filtrate of *L.acidophilus* and *L. casei* against pathogenic microorganisms demonstrated in the table (4). Filtrate of *L.acidophilus* and *L. casei* was concentrated to three folds by the oven. The one - fold concentrated filtrate of LAB gave good inhibitory effect but the effect of two fold was better, while the three fold concentration showed a highest inhibitory effect after 24hr. incubation because all the inhibitory substances were concentrated, zone diameter of *L.a* against *P. aeruginosa*, *E. coli*, *Sal. typhimurium*, *Staph. aureus*, and *C. albicans*, reached to 31, 17, 23, 24 and 27 mm, respectively, and *L.c* has zone diameter reached to 29, 21, 23, 21 and 25 mm, against them, respectively,.

**Table (4):** Inhibitory Effect of *L.acidophilus* and *L.casei* concentrated filtrate against some pathogenic microorganisms estimated by the diameter of inhibition Zone (mm).

| Microorganism | Concentrated | Inhibition Zone Diameter (mm) | |
|---|---|---|---|
| | | ***Lb.a*** | ***Lb.c*** |
| *P. aeruginosa* | **One-fold** | 26 | 25 |
| | **Two-fold** | 29 | 27.5 |
| | **Three-fold** | 31 | 29 |
| *E.coli* | **One-fold** | 13 | 17 |
| | **Two-fold** | 14 | 20 |
| | **Three-fold** | 17 | 21 |
| *Sal. typhimurium* | **One-fold** | 20 | 18 |
| | **Two-fold** | 21 | 20 |
| | **Three-fold** | 23 | 23 |
| *Staph.aureus* | **One-fold** | 18 | 16 |
| | **Two-fold** | 21 | 18 |
| | **Three-fold** | 24 | 21 |
| *C. albicans* | **One-fold** | 20 | 20 |
| | **Two-fold** | 26 | 23 |
| | **Three-fold** | 27 | 25 |

**Inhibitory Activity of S-Layer Proteins Isolated from *Lactobacillus acidophilus* and *lactobacillus casei* on Pathogenic Organisms:**

To evaluate the inhibitory effect of S-layer proteins of *L.acidophilus* and *L.casei*, well diffusion method was used. S-layer protein lacks the ability to inhibit the growth of pathogenic organisms, and this agrees with Johnson-Henry *et al*. (2007) who observed the absence of any bactericidal activity of S-layer protein isolated from *Lactobacillus helveticus* against *E. coli*.

**Conclusions:**

The S-layer protein detected in different species of *Lactobacillus* isolated from different sources, it had been appeared that its molecular weight was different according to the species.

*L. acidophilus* and *L. casei* have inhibitory effects against pathogenic microorganisms (*P. aeruginosa*, *E. coli*, *Staph. aureus*, *Sal. typhimurium* and *C. albicans*). S-layer proteins don't have antimicrobial activity.

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## ARABIC SUMMARY

**تقييم فعالية البروتينات السطحيه ورواشح بكتريا حامض اللاكتيك ضد بعض الأحياء المجهريه الممرضه داخل الزجاج**

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أجريت هذه الدراسة لتقييم فعالية البروتينات السطحيه المعزوله من بكتريا حامض اللاكتيك العصويه بالمقارنة مع فعالية راشح البكتريا الخام المركز ضد بعض الاحياء المجهريه الممرضه وضد خطوط الخلايا السرطانيه داخل الزجاج.

أستخدمت (12 ) عزله من بكتريا حامض اللاكتيك المعزوله من الخل و حليب البشر و حليب البقر و اللبن و المهبل للكشف عن وجود البروتينات السطحيه بطريقة Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) , ثم عزلت البروتينات بواسطة قطع حزمة البروتين السطحي ومعاملتها مع guanidin hydrochloride(G-HCl) تركيز 6 عياري لاسترجاع البروتين من الهلام. قدرت الاوزان الجزيئيه للبروتينات وكانت تتراوح ما بين (37- 63 kDa) حسب اختلاف انواع بكتريا حامض اللاكتيك, كذلك حسبت تراكيز البروتينات السطحيه بأستخدام عده تعتمد في آلية عملها على طريقة البيوريت. و تم أختيار العزلتين *Lactobacillus acidophilus* و *Lactobacillus casei* أعتمادا على الوزن الجزيئي و تركيز البروتين.

حددت الفعاليه التثبيطيه لبكتريا *L. acidophilus* و *L.casei* ضد الاحياء المجهريه الممرضه *Pseudomonas aeruginosa* و *Esherichia coli* و *Staphylococcus aureus* و *Salmonella typhimurium* و *Candida albicans* على وسط MRS الصلب والسائل, وسجلت اقل فعالية تثبيطيه على الوسط الصلب مقارنة بالوسط السائل الذي أعطى أفضل فعالية تثبيطية و أظهر تأثير تثبيطي أكثر ضد بكتريا *P. aeruginosa* أذ وصل القطر التثبيطي الى 24 و 22 ملم للعزلتين على التوالي. و كان التاثير التثبيطي لبكتريا *L.acidophilus* اقوى من التاثير التثبيطي لبكتريا *L. casei* ضد معظم الاحياء المجهريه المستخدمه. و عند دراسة الفعاليه التثبيطيه للبروتينات السطحيه لوحظ ان تلك البروتينات لا تمتلك تأثير تثبيطي ضد الاحياء المجهريه.