

The effect of surlactin produced by *Lactobacillus acidophilus* on eye infectious bacteria in rabbits

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Abstract:

Twenty five vaginal swabs from outpatients' healthy women were collected from Kamal Al-Samarai Hospital, Baghdad, to isolate and identify of *Lactobacillus acidophilus*. Three isolates were diagnosed as *L. acidophilus* which represents 15% of the total number of lactic acid bacterial (LAB) isolates; other LAB types represent 65% (20 isolates). The ability of *L. acidophilus* to produce surlactin was detected after measuring its biological activity to inhibit the adhesion of biofilm formed by *Pseudomonas aeruginosa* to surfaces using test tube method. It was found that all isolates were able to produce surlactin but the activity of surlactin was varying in each isolate. Surlactin produced by isolates 1 and 13 was the most effective. Biological applications of surlactin were studied by inhibiting the adhesion of pathogenic *P. aeruginosa* producing biofilm on contact lenses. In this study the surlactin has the ability to inhibit the adhesion up to 60% and 55% for isolates 1 and 13 respectively and does not have an antibacterial activity. Surlactin showed an ability to treat the infection in rabbits' eyes with *P. aeruginosa* while it did not show this ability against *Staphylococcus aureus*. Additionally, it prevented the infection with *P. aeruginosa* when administrated to rabbits' eyes inoculated with these bacteria only, while it showed no effect against *S. aureus*.

Key words: *Lactobacillus acidophilus*, Surlactin, Eye infections, Rabbit.

Introduction:

Infection of the eye leads to conjunctivitis, keratitis, endophthalmitis and other infections which are responsible for increase incidence of morbidity and blindness worldwide[1]. Das *et al.* (2003) found that *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* as most frequent bacteria in nosocomial ocular infection[2]. *Lactobacilli*, as probiotic agents, are believed to interfere with pathogens by different mechanisms; one of its mechanisms is biosurfactant production[3]. Biosurfactants, a structurally diverse group of surface active molecules synthesized by microorganisms especially those found

as normal flora in the gastrointestinal tract, urogenital tract, the skin and the eye. They interfere with substances grouped on surfaces especially moist and air exposed surfaces as a result they remove those groups, break them and may take their places, therefore; they were used in many industries and medicine to reduce infection and preserved vitality of some substances[4]. Biosurfactants produced by *Lactobacillus* spp. called surlactins (surface lactins) are considered the most important biologically active substances because of their low toxicity and the ability to biodegrade many substances and have an importance medical application to

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reduce microbial infection[5]. *Lactobacillus acidophilus*-derived surlactin have multifunctional substances: as antimicrobial, antitumor, antimutagenic agents and immunomodulators[6]. The use of biosurfactants from probiotic bacteria as antimicrobial and/or anti-adhesive agents has been studied before and their ability to inhibit adhesion of various microorganisms isolated from explanted voice prostheses has been demonstrated[7]. The present study is aimed to enlighten the influence of *L. acidophilus*-derived biosurfactant as antimicrobial and/or anti-adhesive agents and their ability to inhibit adhesion of pathogenic bacteria causing eye infection *in vitro* and *in vivo* (rabbits' eyes).

Materials and Methods:

Bacterial isolates and Culture conditions:

A total of 25 vaginal swabs were obtained from healthy premenopausal women in Kamal Al-Samarai Hospital, Baghdad for the isolation and identification of *L. acidophilus* from April 2007 to December 2007. These swabs were stained by Gram stain and examined microscopically, cultured on selective media Man-Rogosa-Sharp agar (MRS) with 5-10% CO₂ at 37°C (for Lactic acid bacteria isolation). Then growing colonies cultured on MRS agar containing 1%CaCO₃, the ability to form a clear zone around the colonies due to the acid produced by isolates which dissolve the CaCO₃ considered as *Lactobacillus* spp.)[8]. To identify the *L. acidophilus* from other LAB bacteria, the growing colonies cultured on MRS agar containing 1%CaCO₃ were diagnosed according to the biochemical tests and carbohydrates fermentation [9].

Target pathogenic bacteria: *Staphylococcus aureus* (Gram positive bacteria) and *Pseudomonas aeruginosa*

(Gram negative bacteria) which were isolated from wound infections were obtained from Al-Nahrain University, College of Science, Biotechnology department.

Rabbits: Four local and albino from either sex, (6-8) months of age weighing approximately (1.5-2 Kg) were obtained from the National Center for Drug Control and Research, Baghdad.

Contact lenses: synesthetic soft contact lenses (By Fusion™, USA) were purchased from a local pharmacy.

Biosurfactant production:

Activated culture of *L. acidophilus* (selected isolates) was inoculated in MRS broth for 18 hours at 37°C with (5-10% CO₂) and then centrifuged at 6000 round per minute (rpm) for 30 min at 4°C. One ml of the precipitated bacterial cells were inoculated in 25 ml of MRS broth and incubated in the same conditions with shaking at 160 rpm for 18hrs in order to reach the logarithmic phase. The cells were harvested by centrifugation at 6000 rpm for 30 min at 4°C. The precipitate was washed twice with Phosphate Buffer Saline (PBS) and was suspended for 2hrs at 25°C with light stirring for biosurfactant production. Subsequently, the suspension was centrifuged at the same speed for 10 min at 4°C to remove bacterial cells the remaining supernatant liquid was filtered through 0.22µm pore-size filter (Millipore, Bedford, MA, USA) as in Velraeds *et al.*[10].

Biofilm formation inhibition:

In order to form a biofilm, 5 ml of nutrient broth with and without *P. aeruginosa* incubated at 37°C for 48hrs, the content of the tube were discarded carefully and 1% of crystal violet (Fluka) was added to the tube for 15 min, then removed and dried in room temperature (25°C). A biofilm formation as a layer on the inner surface of the tube was noticed by

naked eye. Measuring the biological activity of surlactin to inhibit the adhesion of biofilm formed by *P. aeruginosa* to surface using test tube method was performed according to a modified method of Christensen *et al.*[11]. 250µl of prepared surlactin was added to 5 ml of activated culture of *P. aeruginosa* for 18hrs. The combination was incubated at 37°C for 48hrs, and then the same previously mentioned procedure was done. The results were compared with the positive and negative controls.

Antibacterial activity:

The antibacterial activity of surlactin was tested by the agar diffusion method according to Nathan *et al.*[12]. Nutrient agar (Biolife) inoculated with 0.1ml of (1×10^5 CFU/ml) of activated pathogenic bacteria (*S. aureus* and *P. aeruginosa*) by diffusion method and 3 replica plates were made for each isolate. Three holes of 3mm in diameter were made with equal distance using sterilized cork borer. Equal volumes of 100µl of surlactin, primary filtrate of *L. acidophilus* and chloramphenicol (30µg) (Difco) as positive control were added into the holes. The plates were incubated at 37°C for 24hrs. Results were observed by the formation of inhibition zones around the holes.

Anti-adhesion ability to contacted lenses (in vitro):

Inhibition of pathogenic bacterial adhesion to the contact lenses caused by the effect of surlactin: The method of Kamil, (2005) was used. Ten ml of nutrient broth containing surlactin (500µg/ml) was inoculated with 0.1 ml of (1×10^5 CFU/ml) of activated bacterial growth of *P. aeruginosa*. The culture was added to sterilized contact lenses at 37°C for 48hrs, and then washed with sterilized distilled water. After drying at room temperature, they were stained with 1% crystal violet for 15 min. Then the lenses rewashed from

extra dye. The results were observed by naked eyes in comparison with the control (contact lenses and bacterial suspension without surlactin). Absorbency was measured at 550nm to determine the growth intensity of each case[13].

Anti-adhesion ability to pathogenic bacteria (in vivo):

The effect of surlactin on bacteria causing eye infection in rabbits' eyes (*in vivo*): The procedure of Stern *et al.*, (1982) was used [14]. Four rabbits divided into 2 groups (group 1 and group 2), each group consists of a male and a female. Two injection samples were prepared:

Sample A: Aliquot of 0.5 ml of nutrient broth was mixed with 0.5 ml PBS and 0.1 ml of bacterial suspension (*P. aeruginosa* and *S. aureus*).

Sample B: Aliquot of 0.5ml of surlactin was mixed with 0.5 ml nutrient broth and 0.1 ml of (1×10^5 CFU/ml) bacterial suspension (*P. aeruginosa* and *S. aureus*). The group 1 of rabbits was inoculated with 0.1ml of sample A, group 2 was inoculated with 0.1 ml of sample B (right eye with sample containing *P. aeruginosa* and the left eye with sample containing *S. aureus*). Results were calculated after 24hrs of inoculation. Then group 1 was administrated with 0.1 ml of surlactin and the results were noticed daily.

Statistical analysis: A complete randomized design (CRD) was used. Least significant differences (LSD) of the means were calculated, means were compared at probability of ≤ 0.05 [15].

Results and Discussions:

Isolation of Lactobacillus acidophilus: From 25 vaginal swabs, 20 isolates represents (80%) were able to grow on the selected MRS containing 1% of CaCO₃. They were numbered as 1, 2, 3 to20. These results were agree with Reid (2001)[16], who proved that

Lactobacillus spp. are dominated over other bacterial types that comprise

normal vaginal flora in women as shown in figure 1.

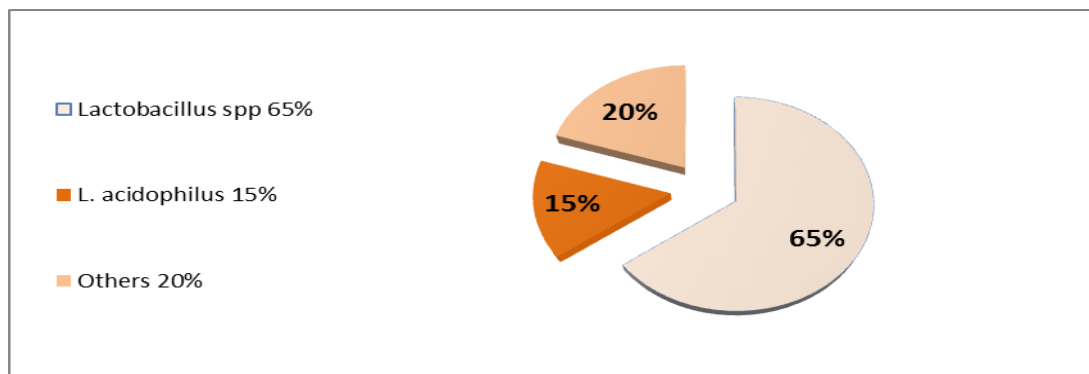


Figure 1: The percentage of bacterial types in vaginal isolates.

Three isolates only represents 15% were identified as *L. acidophilus* (the isolates number 1, 9 and 13). The biochemical tests and carbohydrates fermentation according to Holt and Krieg [9] were performed as shown in Table 1.

Detecting the ability of *L. acidophilus* to produce surlactin:

The ability of the 3 isolates that diagnosed to be as *L. acidophilus* to produce surlactin was detected by inhibiting the adhesion of biofilm produced by the target bacteria (*P. aeruginosa*) in test tubes method. All the 3 isolates were having this ability in different degree. The isolates number 1 and 13 were potent than the

isolate number 9. These results were agree with Boris *et al.*[17] as they mentioned that the biosurfactant produced by *L. acidophilus* is the most effective in inhibiting the adhesion of pathogenic bacteria in comparison to other types. The isolates 1 and 13 were chosen for extraction of surlactin during the stationary phase of bacterial growth by precipitating the cells of the 2 isolates after 18hrs of growth in MRS broth and washing them with PBS to get rid of logarithmic phase products such as bacteriocins, hydrogen peroxide and others. Then it was filtered by 0.22 μ m pore-size filter.

Table 1: The biochemical tests and carbohydrates fermentation for identification of *Lactobacillus acidophilus*.

Isolates	L.1	L.2	L.3	L.4	L.5	L.6	L.7	L.8	L.9	L.10	L.11	L.12	L.13	L.14	L.15	L.16	L.17	L.18	L.19	L.20	
Growth in Litmus milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 15°C	-	-	-	-	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-
Growth at 45°C	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+
Growth at nutrient medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrate Fermentation																					
Fructose	+2	+1	+1	+1	+1	+2	+1	+1	+2	+1	+2	+1	+2	+1	+1	+2	+1	+2	+1	+1	+1
Sucrose	+1	+1	+1	+2	+1	+1	+1	+1	+1	+2	+1	+1	+1	+1	+2	+1	+1	+1	+1	+1	+1
Lactose	+4	+1	+2	+1	+1	+1	+2	+2	+4	+1	+1	+1	+4	+2	+1	+1	+1	+1	+1	+1	+1
Maltose	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1	+1
Mannitol	-	+1	+1	-	-	+1	+1	+1	-	+1	+1	+1	-	+1	+1	-	+1	+1	+1	+1	-
Raffinose	+1	-	-	-	-	-	-	-	+1	-	-	-	+1	-	-	-	-	-	-	-	-
xylose	-	+2	+1	+1	+1	-	+2	+1	-	-	-	+1	-	+2	+1	+1	-	-	+1	+1	

+ = Positive result, - = Negative result, Numbers= No. of days to change the color

The biological and medical application of surlactin:

1- Anti bacterial activity of surlactin (*in vitro*):

The primary filtrates of *L. acidophilus* cultivated in MRS broth for 18hrs had an antibacterial activity against the tested bacteria (*S. aureus* and *P.*

aeruginosa) as the filtrate contains the products of logarithmic phase (bacteriocins, hydrogen peroxides and others), while surlactin extracts of the isolates 1 and 13 had no effect against them as shown in figure 2

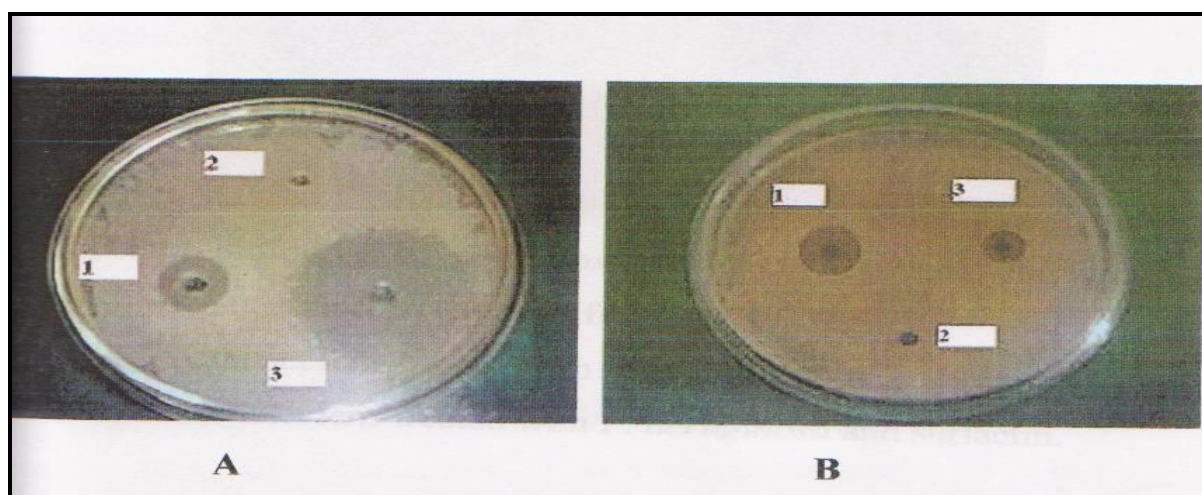


Figure 2: Antibacterial activity of *L. acidophilus* extract (isolate no.1) against the growth of A- *S. aureus* and B- *P. aeruginosa*.

1= primary filtrate of *L. acidophilus* (after 18hrs growth in MRS broth), 2= surlactin, 3= positive control (chloramphenicol)

These results (Table 2) indicates that surlactin lack the ability to inhibit the growth of pathogenic bacteria which, agrees with Velraeds *et al.*[10] as they noticed the absences of any antibacterial activity of surlactin against pathogenic bacteria and

Candida albicans even when its concentration reached 1000µm/ml. These results were also confirmed by Walencka *et al.*[18], in which that the antimicrobial activity of biosurfactants have not been observed.

Table 2: Diameter of inhibition zones caused by the primary filtrate of *L. acidophilus*, surlactin and chloramphenicol on tested bacteria

Bacterial isolates	Diameter of inhibition zone (mm)		
	Primary filtrate of <i>L. acidophilus</i>	Surlactin	Chloramphenicol
<i>P. aeruginosa</i>	11±0.3*	0.0	6±0.5*
<i>S. aureus</i>	13±0.4*	0.0	7±0.6*

*Values are the mean of 3 replicates ± S.E.

2- Inhibition of pathogenic bacterial adhesion to the contact lenses:

The result of this test showed inhibition in the ability of *P. aeruginosa* to adhere to the contact lenses when treated with purified

surlactin for both isolates (1 and 13), differences in crystal violet intensity were noticed (less intensity) in comparison to negative control (lenses not treated with surlactin) as shown in figure 3.

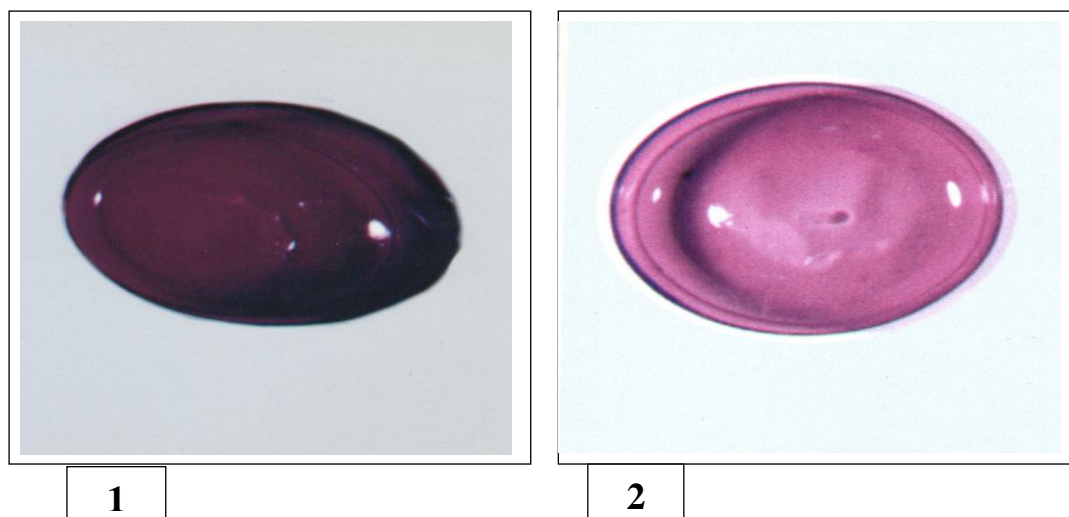


Figure 3: Inhibition of *P. aeruginosa* adhesion to contact lenses using surlactin extracted from isolate 1

- 1= Contact lenses treated with *P. aeruginosa* only (control)
- 2= Contact lenses treated with *P. aeruginosa* and surlactin.

Growth intensity of *P. aeruginosa* was reduced to 60% when treated with surlactin extracted from isolate 1 and 55% for the isolate 13 as shown in figure 4. These results were agree with Kamil [13], who stated that surlactin

extracted from *L. acidophilus* had a good activity in removing biofilm formed by *Staphylococcus epidermidis* from contact lenses[13]. The difference in surlactin activity to inhibit the adhesion of *P. aeruginosa* in contact

lenses and glass tubes was due to the chemical composition of those substances affecting the ability of bacterial cells to adhere to their surfaces.

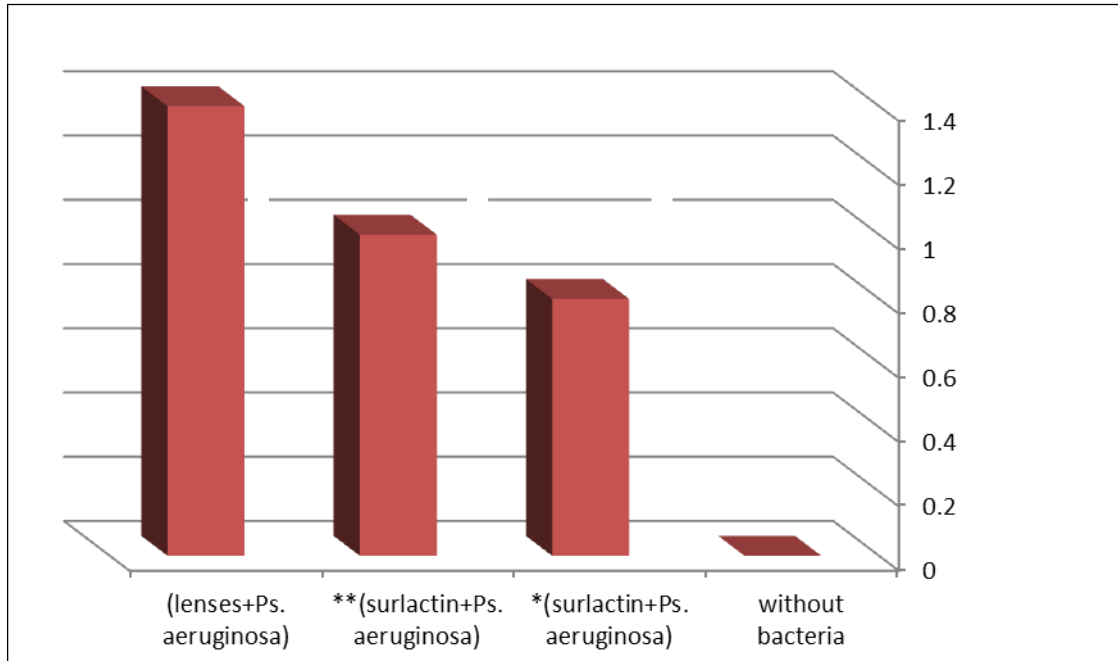


Figure 4: The effect of extracted surlactin on inhibiting the adhesion of *P. aeruginosa* in nutrient broth containing contact lenses. *Surlactin extracted from isolate 13
 **Surlactin extracted from isolate 1

3- The effect of surlactin on bacteria causing eye infection in rabbits' eyes (*in vivo*):

The group 1 was inoculated with sample A, showed swollen, semi closed eyes with red lid filled with pus after 24hrs of the injection as shown in figure 5.

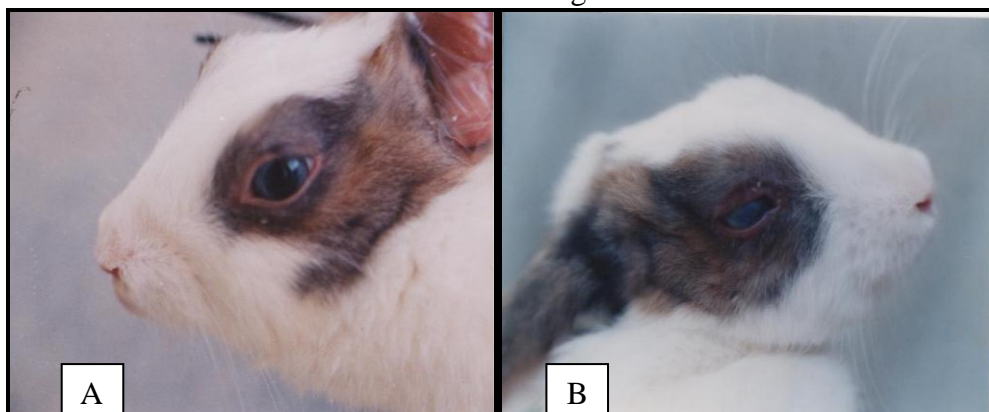


Figure 5: Infection of rabbits' eye after inoculation with *P. aeruginosa* and PBS. A- Before inoculation, B- After inoculation.

When group 1 administrated with surlactin, rabbits' eye infected with *P. aeruginosa* showed a noticed recovery, and full cure occurred after 72hrs of administration (Fig 6-A), while the eye infected with *S. aureus* did not show any recovery and the infection persisted even after one week of the

surlactin administration and increasing the dose (Fig 6-B). These results were agree with Rodrigues *et al.*[19] who confirmed that biosurfactants had inhibitory effect on bacterial adhesion and also biofilm formation.

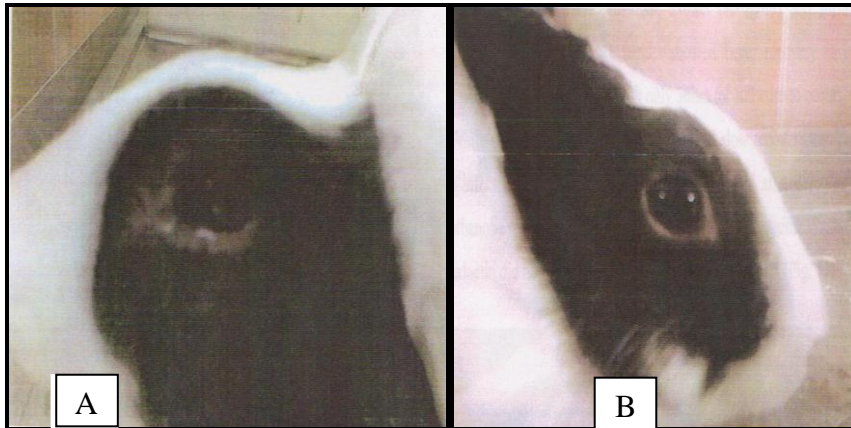


Figure 6: Appearance of rabbits' eye after administration of surlactin extract.

A- Full cure from *P. aeruginosa* after 36hrs of administration

B- Persistence of infection with *S. aureus* after 7 days of administration.

The group 2 was administrated with sample B, did not show any infection or eye redness after 24hrs of administration with sample B containing *P. aeruginosa*. This result could be explained by that the biosurfactant might contain signaling factors that interact with host and/or bacterial cells, leading to the inhibition of infection. These results agree with Falagas and Markis [20] who stated that previous adsorption of biosurfactant can be use as a preventive strategy to delay the onset of pathogenic growth on medical implant materials. In a study by Tahmourespour *et al.* [21] found that biosurfactant produced by *L. acidophilus* was able to interfere in the adhesion and biofilm formation of the *Streptococcus mutans* to glass slide. Several properties *S. mutans*

cells (the surface properties, biofilm formation, adhesion ability and gene expression) were changed after *L. acidophilus* derived biosurfactant treatment. It is also concluded that biosurfactant treatment can provide an optional way to control biofilm development and suggest that the prepared biosurfactant may interfere with adhesion processes of *S. mutans* to teeth surfaces. Meanwhile administration with sample B containing *S. aureus* show eye redness after 24hrs of administration, because the surlactin have no antibacterial activity which agree with Velraeds *et al.* [10]

Conclusion:

All *Lactobacillus acidophilus* isolates 1, 9 and 13 showed the ability to produce surlactin. Surlactin extracted from isolates 1 and 13 were the most effective in its biological activity, which was determined by its ability to inhibit the biofilm formation produced by *P. aeruginosa* using test tube method as well as in contact lenses but lack this ability against *S. aureus*. Administration of surlactin to infected rabbits' eye with *P. aeruginosa* showed a full cure after 36hrs and persistence infection with *S. aureus*. The surlactin has no antibacterial activity against tested pathogenic bacteria (*S. aureus* and *P. aeruginosa*). In conclusion the surlactin may have a potential application as anti-adhesive agent but not as antibacterial agent against *P. aeruginosa*.

References:

- Juarez-Verdayes MA, Reyes-Lopez MA, Cancino-Diaz ME, *et al.* 2006 Isolation, vancomycin resistance and biofilm production of *Staphylococcus epidermidis* from patients with conjunctivitis, corneal ulcers, and endophthalmitis. *Rev. Latinoam. Microbiol.*; 48(3-4): 238-46.
- Das A, Dey AK, Agarwal PK, Mazumdar AK, Mazumdar S, and Chatterjee SS 2003 Nosocomial ocular infection – a prospective study. *J. Indian. Med. Assoc.*; 101:490-92.
- Rodrigues L, Banat IM, Teixeira J, and Oliveria R 2006 Biosurfactants: Potential application in medicine. *J. Antimicrob. Chemother.*; 57: 609-618.
- Van Hoogmoed CG, Van Der Kuijl-Booij M, Van Der Mei HC and Busscher HJ 2000 Inhibition of *Streptococcus mutans* NS adhesion to glass with and without a salivary conditioning film by biosurfactant-releasing *Streptococcus mutans* strains. *Appl. Environ. Microbiol.*; 66(2):659-63.
- Rhee, C and Park, H 2001 Three glycoproteins with antimutagenic activity identified in *Lactobacillus plantarum* KLAB21. *Appl. Environ. Microbiol.*; 67(8):3445-49.
- Eijsink V, Middelhoven P, Skeie M, Brurberg M and Nes I 1998 Comparative studies of class II bacteriocins of lactic acid bacteria. *Appl. Environ. Microbiol.*; 64(9):3275-81.
- Rodrigues L, Mei HC, Teixeira J, and Oliveira R 2004 Influence of Biosurfactants from Probiotic Bacteria on Formation of Biofilms on Voice Prostheses. *Appl. Environ. Microbiol.*; 70(7): 4408-10.
- Al-Kassab AJO and Al-Khafaji ZM 1992 Effect of different factors on the antagonism of Enteric *Lactobacilli* with Diarrheal Enterbacteria. *Iraqi J. Agricul. Sci.*; 23(1): 18-26. (in Arabic)
- Holt E and Krieg N 1986 Bergy's Manual of Systemic Bacteriology. Vol.2, Williams and Wilkins, London.
- Velraeds M, Van der Belt-Gritter B, Van der Mei H, Reid G and Busscher H 1998 Interference in initial adhesion of uropathogenic bacteria and yeasts to silicon rubber by a *Lactobacillus acidophilus* biosurfactants. *J. Med. Microbiol.*; 47: 1081-1085.
- Christensen GD, Simpson WA, Bison AC and Beachey EH 1982 Adherence of slim-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect. Immunol.*; 37(1): 318-326.
- Nathan P, Law E, Murphy D and Macmillan BG 1978 A laboratory

- method for selection of topical agents to treat infected burn wounds. *J. Burns*; 4 (issue 3): 177-187.
13. Kamil FH 2005 Extraction and purification of surlactin produced by *Lactobacillus acidophilus*. *PhD. Thesis*, College of Science, Al-Mustansirya University, Baghdad-Iraq.
 14. Stern GA, Weitzenkron D and Valenti J 1982 Adherence of *Pseudomonas aeruginosa* to the mouse cornea. Epithelial Vs. Stroma. *Arch. Ophthalmol.*, 100(12): 1956-58.
 15. Gomez KA and Gomez AA 1984 Statistical procedures for Agricultural research, 2nd., *John Wiley and Sons*.
 16. Reid G 2001: probiotic agents to protect the urogenital tract against infection. *Am. J. Clin. Nutr.*, 73(2 suppl.): 437-443.
 17. Boris S, Suárez JE, Vázquez F, and Barbés 1998 Adherence of human vaginal *Lactobacilli* to vaginal epithelial cells and Interaction with Uropathogens. *Infect. Immunol.*, 66(5): 1985-89.
 18. Walencka E, Rozalska S, Sadowska B and Rozalska B 2008 The Influence of *Lactobacillus acidophilus*-Derived Surfactants on *Staphylococcal* Adhesion and Biofilm Formation. *Folia Microbiol.*; 53(1), 61-66.
 19. Rodrigues L, Banat IM, Teixeira J and Oliveria R 2006 Biosurfactants: Potential application in medicine. *J. Antimicrob. Chemother.*; 57: 609-618.
 20. Falagas ME and Markis GC 2009 Probiotic bacteria and biosurfactant for nosocomial infections control. A hypothesis. *J. Hosp. Infec.*; 71: 301-306.
 21. Tahmourespour A, Salehi R, Kermanshahi RK 2011 *Lactobacillus acidophilus*-derived biosurfactant effect on gtfb and gtfc expression level in *Streptococcus mutans* biofilm cells. *Brazil. J. Microbiol.*; 42: 330-339

تأثير Surlactin المنتجة من بكتريا *Lactobacillus acidophilus* على البكتريا المسببة لالتهاب العيون في الأرانب

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الخلاصة:

تم جمع 25 مسحة مهبلية من نساء صحيحات من مستشفى كمال السامرائي/ بغداد بهدف عزل وتشخيص بكتريا *Lactobacillus acidophilus*. تم تشخيص 3 عزلات من هذه المسحات كونها *L. acidophilus* والتي شكلت نسبة 15% من مجموع العزلات البكتيرية المنتجة لحامض اللكتيك التي تم الحصول عليها، وكانت نسبة الأنواع الأخرى للبكتريا المنتجة لحامض اللكتيك 65% (20 عزلة تم إجراء اختبار كفاءة عزلات *L. acidophilus* لإنتاج مادة surlactin عن طريق قياس فعاليته الحيوية بتأثيره على الغشاء الحيوي المنتج من بكتريا *Pseudomonas aeruginosa* والتصاقها بالسطوح الملساء بطريقة انابيب الاختبار. ووجد ان العزلات الثلاثة كانت منتجة لمادة surlactin ولكن بفعالية مختلفة لكل عزلة، إذ كان surlactin المنتج من العزلتين 1 و 13 الأكفأ من حيث الفعالية. تم دراسة التطبيقات الحيوية لمادة surlactin عن طريق تثبيط التصاق الخلايا الممرضة *P. aeruginosa* المنتجة للغشاء الحيوي في عدسات العين اللاصقة، إذ وجد أن له قابلية في تثبيط الالتصاق بنسبة 60% و 55% للعزلتين 1 و 13 على التوالي وليس لها قابلية على تثبيط النمو الجرثومي. أظهرت مادة surlactin القدرة على معالجة الالتهابات في عيون الأرانب المصابة ببكتريا *P. aeruginosa* ولم تكن لها القدرة على معالجة لالتهابات في عيون الأرانب المصابة ببكتريا *Staphylococcus aureus*. كما منعت حدوث الأصابة في عيون الأرانب عند حقنها مع بكتريا *P. aeruginosa* بينما لم يكن لها أي تأثير في الحد من الالتهابات عند حقنها مع بكتريا *S. aureus* في عيون الأرانب.