



## Antibacterial and Molecular Docking Analysis of Biosurfactants Produced from *Lactobacillus Acidophilus* Against MDR *Staphylococcus aureus*

Sumod Abdul Kadhem Salman<sup>1</sup>

Balsam Miri Mizher Al Muhana<sup>1</sup>

<sup>1</sup>Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq

<sup>1</sup>Department of Microbiology, College of Veterinary Medicine, Al-Qasim Green University, Iraq

Submitted: July 14, 2025

Revised: July 28, 2025

Accepted: July 28, 2025

### Correspondence

Sumod Abdul Kadhem Salman

[Sumodkadem8@vet.uoqasim.edu.iq](mailto:Sumodkadem8@vet.uoqasim.edu.iq)

Balsam Miri Mizher Al Muhana

[Balsam.mizher@qu.edu.iq](mailto:Balsam.mizher@qu.edu.iq)

**Abstract** Lactic acid bacteria are members of probiotic bacteria and their metabolites as biosurfactants (BS<sub>s</sub>) emerged as great antibiotics alternatives. The current study aimed to produce, purify and evaluate the antibiofilm effect of BS<sub>s</sub> produced by *Lactobacillus acidophilus* isolate against multidrug resistant (MDR) *Staphylococcus aureus*. BS<sub>s</sub> were screened by preliminary tests like emulsification assays, oil spreading test and foaming activity. Purification of BS<sub>s</sub> was done by silica column chromatography, analyzed by FTIR and Gas chromatography–mass spectrometry. Broth macro-dilution method was employed to find Minimum inhibitory concentration (MIC) of BS<sub>s</sub>. Quantitative Reverse Transcriptase Real-Time PCR was to study expression for *fib* gene implicated in biofilm formation of 30 isolates of MDR *S. aureus* before and after treating these isolates with three concentrations of sub-MIC of BS<sub>s</sub>. Emulsification assay for BS<sub>s</sub> was 67%, clear zone (diameter 7 cm) was formed on the surface of petri dish contain oil-water interface. The foaming activity displayed percentage of 60%. FTIR analysis proposed that it composed of glycolipoproteins. GC-MASS showed presence of different biologically compounds as Benzoic acid, n-Hexadecanoic acid, Linoelaidic acid. The MIC value of BS<sub>s</sub> against MDR *S. aureus* isolates was 0.625 mg/mL. The results revealed that 26 (86.6%) of MDR isolates were positive for biofilm production. Only 4 (13.3%) of isolates produced biofilm after treating with sub-MIC of BS<sub>s</sub>. Chemical compounds in BS<sub>s</sub> was exposed to the molecular docking analysis against one of biofilm protein (*fib* protein) of *S. aureus*. Benzoic acid possess good affinity toward *fib* protein (binding energy is 7.5).

**Keywords:** Probiotic, Biosurfactants, MDR, *S. aureus*, biofilm.

©Authors, 2025, College of Veterinary Medicine, University of Al-Qadisiyah. This is an open access article under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>).

**Introduction** Probiotics are non-pathogenic and non-invasive microorganisms that play a vital role in the improvement of host's health when administered in the appropriate amounts (1). Lactic acid bacteria (LAB) including *Lactobacillus acidophilus* (*L. acidophilus*) are considered as a main group of probiotic bacteria (2). *L. acidophilus* had positive effect and caused inhibition in bacteria growth of *Staphylococcus spp.*, *pseudomonas spp.*, *kocurea spp.*, and *Granulicatella spp* (3). Probiotic enhanced growth performance, immune system activity and reduce ammonia levels (4,5). BS<sub>s</sub> is a chemical substances produced from LAB. It is amphipathic molecules with hydrophilic and hydrophobic moieties (6). BS<sub>s</sub> have unique properties such as eco-friendly, high biodegradability, low toxicity and resistance to extreme conditions of temperature and pH, therefore

it can be used in different industrial fields (7). Further, BS<sub>s</sub> have numerous effects like antiviral, antiadhesive, anti- HIV, anti-inflammatory, and antimicrobial activities (8). *Staphylococcus spp.* are widely dispersed in the environment and is primarily discovered as a commensally micro-flora on the skin of animals and birds as well as the mucous membranes (9), because of its ability to establish resistance against wide range of new and old antibiotics. *S. aureus* in nature exist in aggregated communities known as biofilms, and cells within a biofilm demonstrate major resistance to antibiotics and disinfectants (10). Antibiotics resistance is considered as very important global public health because its contributed for 1.27 million worldwide deaths in 2019 (11). So, it's very important to seek an alternative therapeutic agent that would be a



promising effect for control and treatment of varied bacterial infection. So, this study aimed to assessed antibiofilm effect of BS<sub>s</sub> extracted from probiotic bacteria against MDR *S. aureus* isolates.

#### **Materials and methods**

##### **Ethical approval**

The project was approved (1669 in 20/4/2025) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

##### **Source of bacteria**

Standard isolate of *L.acidophilus* was obtain from Al-Amin Center for research and Biotechnology in Al- Najaf Al-Ashraf/ Iraq .Small amounts (0.1 gm) of lyophilized isolate was liquefied in 10 ml of sterilized distill water , gently shaking and then cultured in MRS agar at 37°C for 24 hours. On the other hand, 30 isolates of MDR *S.aureus* were Obtained from laboratory of Microbiology in Veterinary Medicine College/ University of Al- Qadisiyah.

##### **Optimization the medium compositions for biosurfactant production**

MRS broth was modified to obtain an optimum medium for BS production. This modification included the addition of the following materials: yeast extract 5 gm/L, lactose 20 - 30 gm/L and sunflower oil 4% (12).

##### **Biosurfactants Production**

*L.acidophilus* isolate was subcultured in 10 ml modified MRS broth incubated at 37°C for 24 hours. Then the culture was transferred into 500 ml MRS broth and then incubated at 37°C for 96 h. The culture was centrifugaed at 6000 rpm for 25 minutes to eliminate the bacterial cells and then collect the supernatant (13).

##### **Detection of emulsification index**

Two ml of bacterial supernatant was transferred to test tube contain about 2 ml of kerosene then vortexes for 3 minutes and incubated at 25°C overnight. The emulsification index (E24) estimated according to the following equation (14).

$E24 = \text{Total height of emulsion} / \text{Total height of solution} * 100.$

##### **Oil spreading test**

To petri dish contained distilled water, 100 µl of oil was added then the supernatant (50 µl) was also added to the same plate. Oil displacement and clear zone formation around the drop of culture supernatant indicated biosurfactants presence (15).

##### **Foaming activity**

Four ml of supernatant was shaken by hand for two minutes. The foam activity was measured by the following equation (16).

$\text{Foaming} = \text{foam height} / \text{total height} * 100$

##### **Biosurfactants Extraction**

Extraction of the BS was done according to Darvishi *et. al* (17) with some modification. The PH of the supernatant was adjusted to 2 using 6 N HCL and kept overnight at 4°C. After incubation the supernatant was centrifuge at 6000 rpm for 25 minutes and the pellet was mixed with same quantity of chloroform: methanol at ratio 2:1 then strongly mixed and leaved it aside for hours to separate the mixture phases. Three layers were formed after phases separation – the upper layer is the supernatant, the middle one is the BS and the lower is the chloroform: methanol. BS was prudently collected by micropipette and dried for 24 h.

##### **Purification of Biosurfactant by silica column chromatography**

The dried material was dissolved in methanol (1 gm/ 10 ml) to purify it by silica column chromatography with measurements (3.5 x 30 cm) and packed with silica gel (60 mesh). It was full firmly by an incessant flow of methanol then wash the column by methanol. 10 ml of methanol was used to dissolve BS<sub>s</sub> (1 gm) and full in column till more of the mixture is absorbed. This column was eluted by grade of methanol and chloroform extending from (250 ml) of 3:50 (15 ml methanol + 250 ml chloroform), (200ml) 2:50 (20 ml methanol +200 ml chloroform), (100ml) 50:50 (50ml methanol + 50 chloroform) and 100 ml of methanol alone. Then the solution flowing at ratio 20 ml / h and 3 ml from each portion were collected. All portions collected and verified for emulsification activity and surface tension any part hold BS<sub>s</sub> experienced for extra tests (18).

##### **Characterization of purified biosurfactant**

###### **FTIR analysis of Biosurfactants**

The functional groups of BS<sub>s</sub> compounds were determined by. The range was limited at 4000-400 cm-1 with resolution of 4 cm-1 (19). This test was done in chemistry analysis center / Baghdad.

###### **Analysis with Gas chromatography (GC)**

To determine the type and numbers of components found in the extracted BS, GC-MS was used. 1 ml of sulfuric acid – methanol was used to dissolve BSS (10 mg) at 90 °C for 15 hours. After addition of hexane the mixture is moving then the layer of hexane was extracted and sulfuric acid was vaporized. After addition of 1 ml distal water to hexane layer, the mixture was mixed carefully. The fatty acid methyl ester removed with hexane and then examined by GC using helium as carrier of gas on

Shimadzu 17A GC have fused silica capillary column (30m × 0.25 mm, 0.25 μm film thickness) (20).

### Determination of Minimum Inhibitory Concentration (MIC) of Biosurfactants

The MIC of purified BS<sub>s</sub> was measured by the modified broth macro-dilution method. A twofold serial dilution of BS<sub>s</sub> was prepared by dissolving the dried BS<sub>s</sub> in sterile D.W to obtain a concentration range from 5 to 0.320 mg /ml. 500 μL of each dilution is transferred into tubes contain 4ml of sterilized Muller Hinton broth, then inoculated with 500 μL of standardized inoculums of *S. aureus* isolates that compared with 0.5 McFarland standard tube (1.5 ×10<sup>8</sup> cell/ml). The tubes incubated at 37 °C for one day (21).

### Antibiofilm activity of Biosurfactants

Biofilm producer isolates was assessed using the Congo Red Agar (CRA) method. These plates were prepared by using the following components: Congo red stain (0.8 gm/l), brain heart infusion broth (37 gm/l), sucrose (5gm/l) and agar (10 gm/l). Congo red stain was prepared separately as aqueous solution and then autoclaved at 121<sup>0</sup> C for 15 minutes, then the solution was mixed with brain heart infusion agar at 55<sup>0</sup> C. The sugar was mixed with other components. Antibiofilm activity of BS<sub>s</sub> were tested by growing the isolates with and without sub-MIC of BS<sub>s</sub> and then cultured on CRA plates. Plates incubated aerobically at 37<sup>0</sup> C for 18-24 hours. Black colonies with a dry crystalline refer to biofilm producing isolates (22).

### Molecular docking

#### Preparation of ligand molecules

The chemical compounds in purified BS<sub>s</sub> that identified by GC–MS analysis were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), these ligands were prepared using PyRx-Vina Program 0.8 and assembled by using OpenBabel software version 3.1.1 . All these compounds were docked separately against the target fib protein (23).

#### Preparation of target protein

This computational technique was performed to predict how fib protein (biofilm protein) of *S.aureus* and the ligands (BSs) fit together and interact, which can be correlated with antibiofilm activity. This protein was cleaned up by removing alternate conformations, altering the terminal residues, and correcting the bond orders. The proteins were prepared by removing water molecules, then further refined through energy minimization using the PyRx-Vina Program 0.8.

### Molecular docking between ligand molecules and fib protein

All docking and scoring calculation were achieved using PyRx-Vina Program 0.8 with vina space search (Center: X: 19.981, Y: 11.5986 and Z: 11.69.3) (Dimensions Å: X: 26.6637, Y: 29.4532 and Z: 27.8510) for fib protein of *S.aureus*. The crystal structure of fib protein (PDB entry: 7P2M) at a resolution of 1.16 Å and the crystal configuration of *S. aureus* DNA gyrase (PDB entry: 3U2D) at a resolution of 1.85 Å was gotten from Protain Data Bank. A resolution ranging from 1.5 and 2.5 Å is good value for docking tests. It is well-known that the finest score of Root Mean Square Deviation (RMSD) standards must be close to 2 Å with an energy scoreless or equivalent to 7 Kcal /mol (23). The two values were frequently used as measure the validity of the result of molecular docking. The protein-ligand interactions were analysis and visualized by using BIOVIA discovery studio V21.1.0.20298.

### Quantitative Reverse Transcriptase Real Time PCR

This method was done for study the *fib* gene expression which implicated in biofilm formation before and after treatment of the tested bacteria with three sub-MIC concentrations of BS. This gene is normalized by housekeeping gene *rpoB*. Total RNA was extracted using Trizol Reagent (easy-BLUE™ Total RNA Extraction Kit) according to instruction of manufactured company. This extracted RNA was tested by using Nanodrop. RNA was treated with DNase (Promega company, USA) to remove any DNA then it converted to its complementary DNA using the M-MLV Reverse Transcriptase kit. All these process were done rendering to the manufacturer’s directions, primer pairs used in this study were listed in table (1). Master mix of target gene and housekeeping gene was set by using kit (GoTaq qPCR Master Mix Kit) with SYBER green dye amplification in Real Time PCR system. Components of master mix put in qPCR plate strip tubes, vortexes and centerfuged for five minutes then put in CFX96 Real Time system. According to qPCR kit instructions, thermocycler conditions was prepared. Housekeeping gene was used to normalize the expression of target genes. Relative gene expression levels were determined using the ΔΔCT method.

**Table 1:** Primers of Real Time PCR

Primers	Sequence 5'-3'	PCR product	NCBI Reference code
---------	----------------	-------------	---------------------

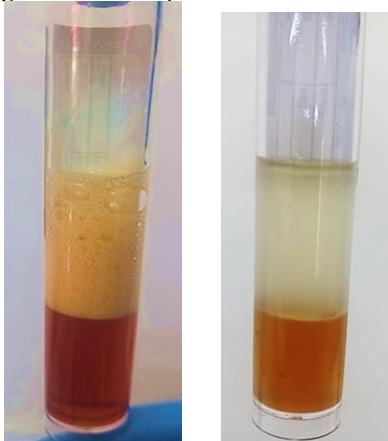
fib gene	F:ACATTCGAATATGG TGCACGTC	142 bp	PP5371 60.1
	R:TTCTGTGTGCACTG ACAGTATG		
rpoB gene	F:CGTGCTCAAATGGA AGTACGTG	77 bp	KU555 590.1
	R:GGTCCCTCAGGTGT TTCAATTG		

**Results**

Lyophilized isolate was culture in MRS agar as shown in figure (1). The isolate was subculture in MRS broth with three inducer components; yeast extract 5 gm/L, lactose 25 gm/L, sunflower oil 4%. The isolate that grown with these components give 3 g/l compare with 1.4 g/l when the same isolate was grown on control medium (the same broth without additions). The emulsification activity of *L.acidophilus* isolates was 67 % as shown in figure (2-A).



**Figure 1:** *L.acidophilus* isolates on MRS agar

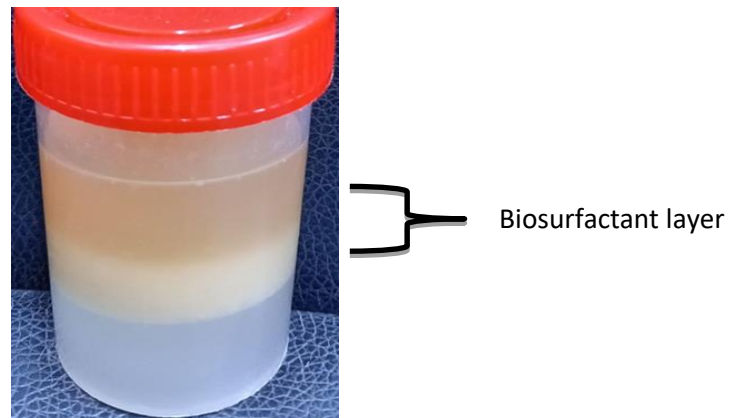


**Figure 2:** Emulsification assay and Foaming activity of *L. acidophilus* isolate A: -Emulsificationassays B: - Foaming activity

Also the foaming activity of *L.acidophilus* isolate was found 60% (figure 2-B). Foaming production is an important test to examine BS<sub>s</sub> production (24). Formation of a clear zone with a diameter 7 cm because of diffusion of BS drop on the surface of petri dish contain oil-water interface and reduce the surface tension.

**Extraction of Biosurfactants**

Bacterial supernatant was extracted using equal amounts of chloroform: methanol (2:1) as mentioned by (17) to obtain partially purified BS<sub>s</sub>. BS<sub>s</sub> is appeared in the middle layer as shown in figure (3) which is can separated out by plastic pipette and then purified after evaporating overnight in the oven.



**Figure 3:** Extraction of Biosurfactant by chloroform: methanol.

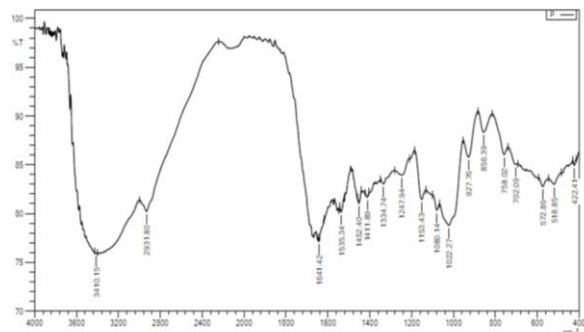
Silica column chromatography was used in this study to purify BS<sub>s</sub> as shown in figure (4). Silica gel column (3.5 × 30 cm) was loading by crude BS which liquefied by chloroform. The eluted parts collected, the surface tension and emulsification activity for any fraction tested.



**Figure 4:** Extraction of Biosurfactant by silica column chromatography technique

**Characterization of produced Biosurfactants  
FTIR analysis**

The FTIR spectrum of purified PS was used to identify the functional groups of the components based on the peak value in the region of infrared radiation. The FTIR spectra of BS were studied in the range of 400–4000  $\text{cm}^{-1}$  region (Figure 5). FTIR analysis showed that the observed peak at about 3410  $\text{cm}^{-1}$  is due to the stretching vibration of the hydroxyl group this might be attributable to the polysaccharide O-H groups. The peak at 2931.8  $\text{cm}^{-1}$  represented compound have C-H bond indicating the aliphatic chains of lipid. The peak near 1641  $\text{cm}^{-1}$  is the absorption peak of the C=N stretching vibration (imine-oxime). The absorption peaks around 1535.3  $\text{cm}^{-1}$  is the distinctive absorption peak of strong N-O stretching vibration of nitro-compound. The band at 1452.4  $\text{cm}^{-1}$  is corresponding to the medium C-H bending of alkane. The peaks at 1334.74  $\text{cm}^{-1}$  was assigning to medium OH bending of carboxylic acid. The band at 1247.94  $\text{cm}^{-1}$  correspond to the strong CO stretching aromatic ester. The other peaks at 1080.14 and 1022.27  $\text{cm}^{-1}$  are due to the strong C=O stretching vibration of alkyl groups. The peak around 758.02–702.09  $\text{cm}^{-1}$  is assigned to the strong C-H bending. According to these finding, there are lipid, sugars, and peptides, the molecular composition of BS<sub>s</sub> is glycolipopeptides. These observations propose that our substances composed of glycolipoproteins. Hydrophobic part of BS<sub>s</sub> is comprised of lipid and hydrophilic chain is chiefly composed of sugar.



**Figure 5:** FTIR spectrum analysis of biosurfactant produced from *L. acidophilus*

**Analysis with Gas chromatography–mass spectrometry (GC-MS)**

GC-MS characterization of the purified BSs was done to identify the compounds found in sample. BS produced by *L. acidophilus* showed the presence of different biologically active compounds as shown in Table (2) and Figure (6). According to the results the purified BS contain higher percentages (36.44%) of 9-Octadecenoic acid - peak No. 5, in addition to a smaller proportion of other saturated fatty acid, polyunsaturated fatty acid, monounsaturated fatty acid and unsaturated fatty aldehyde as shown in Table (2).

**Table 2: GC- MASS analysis of Biosurfactants produced from *L. acidophilus***

Nu mb er of pea ks	Reten tio n time (min ute)	Compoun d	Group	a r e a	A re a %
1	49.9 87	Benzoic acid, phenylmeth yl ester	aromati c ester	347254 0	1. 26
2	56.4 91	n- Hexadecan oic acid	carboxy lic acids(sa turated fatty acid)	600420 39	21 .7 3
3	56.7 71	n- Hexadecan oic acid	carboxy lic acids(sa turated fatty acid)	729508	0. 26
4	61.8 63	Linoelaidic acid	polyuns aturated fatty	359396 38	13 .0 1

			acid		
5	62.0 63	9- Octadeceno ic acid	monoun saturate d fatty acid	100673 743	36 .4 4
6	62.2 00	9- Octadeceno ic acid	monoun saturate d fatty acid	491978 35	17 .8 1
7	62.7 21	1Heptadeca necarboxyli c acid	saturate d fatty acid	178021 22	6. 44
8	64.6 12	Linoelaidic acid	polyuns aturated fatty acid	46782	0. 02
9	66.3 10	9,12- Octadecadi enoic acid	monoun saturate d fatty acid	428903	0. 16
10	67.8 47	Cyclopropa neoctanal, 2-octyl-	polyuns aturated fatty acid	761957 7	2. 76
11	68.0 07	cis-9- Hexadecen al	unsatur ated fatty aldehyd e	328915	0. 12



**Figure 6:** GC analysis of purified biosurfactants produce from *L.acidophilus*

**Determination of Minimum Inhibitory Concentration (MIC) of Biosurfactants**

MIC is the lowest concentration of antibiotics that inhibit microorganism growth. Our results exhibited that MIC values of BS against *S.aureus* are 0.625 mg/ml .

**Biofilm Assay:**

Antibiofilm activity of BSs were tested by growing the isolates with and without sub- MIC of BSs then culture in CRA. When the isolates were culturing without BS, 26 (86.6%) of MDR isolates were positive for biofilm, while when the isolates were

grown on the same media with sub-MIC of BSs only 4 (13.3%) of isolates were positive for biofilm production. The biofilm was inhibited and this was indicated by the lack of the dry crystalline black colonies (Table 3).

**Table 3:** Biofilm production by Congo red method, with and without sub-MIC of biosurfactant extracted from *L.acidophilus*

Bacterial isolates	Biofilm production isolates No. (%)
Without biosurfactants	26 (86.6%)
With biosurfactants	4 (13.3%)
X <sup>2</sup>	32.26
P value	<0.0001

**Molecular docking analysis**

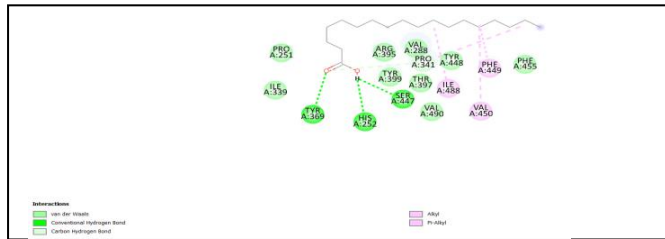
The chemical compounds in purified BS that identified by GC-MS were subjected to the molecular docking toward one of biofilm protein (fib protein) of *S.aureus*. As shown in Table (4) Benzoic acid had a high affinity for fib protein with a binding energy of -7.5 followed by (-6) for n-Hexadecanoic acid, Linoleaidic acid and Cyclopropaneoctanal. The RMSD/UB (Root Mean Square Deviation/Upper Bound) and RMSD/LB (Root Mean Square Deviation/Lower Bound) value of almost all bioactive compounds was (0) which showed the correctness of molecular docking results as mentioned in Table (4).

**Table 4:** The molecular docking results of active compounds in biosurfactant against fib protein of *S.aureus*.

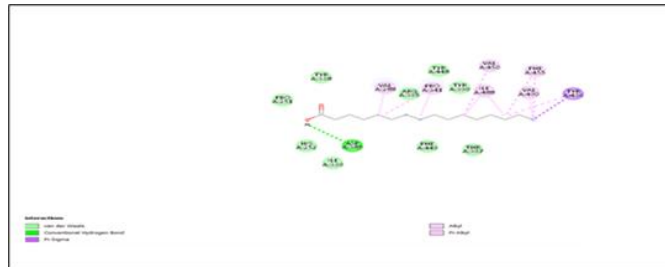
Ligands	Bin ding Affi nity	rms d/ub	rms d/lb
In67_Fib_protein_clear_Benzoic acid	-7.5	0	0
In67_Fib_protein_clear_9	-6.3	0	0
In67_Fib_protein_clear_n-Hexadecanoic acid uff E=57.46	-6	0	0
In67_Fib_protein_clear_Linoelaidic acid uff E=81.58	-6	0	0
In67_Fib_protein_clear_Cyclopropaneoctanal	-6	0	0
In67_Fib_protein_clear_9-Octadecenoic acid uff E=69.17	-5.8	0	0
In67_Fib_protein_clear_1-Heptadecanecarboxylic acid uff E=41.91	-5.5	0	0

In67_Fib_protein_clear_cis-9-Hexadecenal uff E=105.16	-5.2	0	0
---	------	---	---

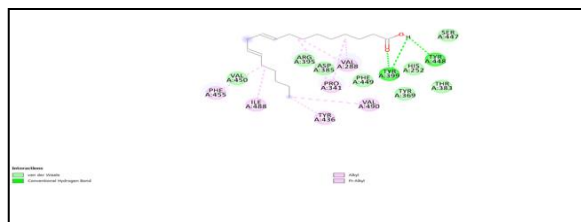
The amino acids that participated in the interaction for each ligand were showed in figure (7 to 10). Benzoic acid interacted with seven amino acids, while n-Hexadecanoic acid interacted with nine amino acids.



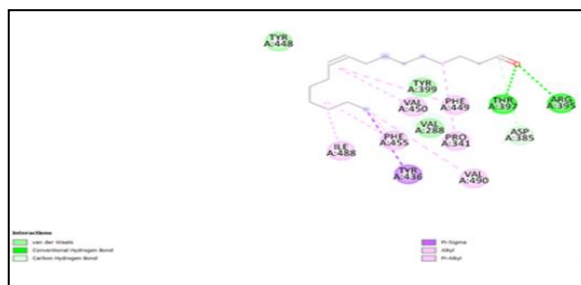
**Figure 7:** Benzoic acid interaction with fib protein of *S. aureus*



**Figure (8):** n-Hexadecanoic acid interaction with fib protein of *S. aureus*.



**Figure 9:** Linoleic acid interaction with fib protein of *S. aureus*



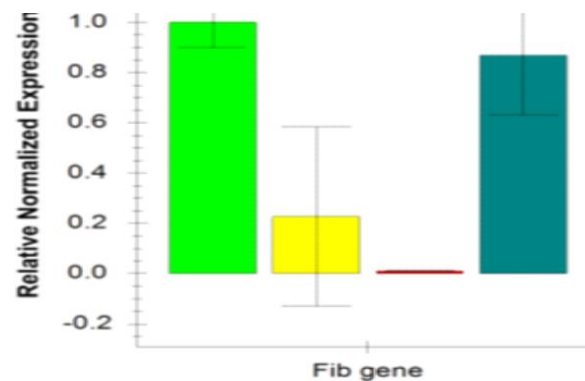
**Figure 10:** Cyclopropanoic acid interaction with fib protein of *S. aureus*

### Effect of biosurfactants on the expression of *S. aureus fib* gene associated with biofilm production

The *fib* gene expression was assessed before and after treatment of *S. aureus* isolates with three concentrations of sub-MIC of BS<sub>s</sub> (320, 160, 80 µg/mL) as shown in table (5) by standardization with expression of RNA polymerase β subunit gene (*rpoB*). Figure (11) shows that BS<sub>s</sub> produced by *L. acidophilus* effect on the expression of *fib* genes in *S. aureus* isolates in concentration dependent manner compared with control. In the concentrations 80 µg/mL and 160 µg/mL, no significant difference ( $P > 0.05$ ) was observed in the inhibition of the *fib* gene while at the concentration 320 µg/ml, the expression level of this gene was significantly decreased ( $P < 0.05$ ).

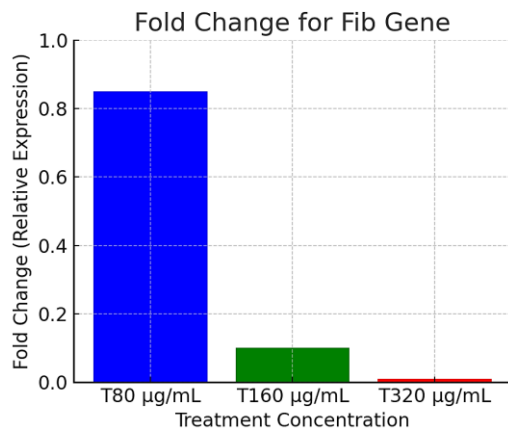
**Table 5:** Concentrations of sub-MIC of biosurfactants against expression of *fib* gene of *S. aureus* isolates

Treatment No.	Name
T1	320 µg/ml supplemented with sugar
T2	160 µg/ml supplemented with sugar
T3	80 µg/ml supplemented with sugar
Control	Bacterial broth supplemented with sugar



**Figure 11:** The effect of Biosurfactants produce from *L. acidophilus* on the expression of *fib* gene of *S. aureus*

The fold change in gene expression was calculated relative to control samples as shown in figure (12).



**Figure 12:** Fold change analysis for the *fib* gene of *S. aureus* treated with different concentrations of Biosurfactants

### Discussion

Culture conditions of *L.acidophilus* were optimized to produce BS<sub>s</sub> with specific characteristic and appropriate amounts. In this study *L.acidophilus* isolate was culture in aerobic conditions but due to this species is facultative anaerobic other study was grown it in anaerobic condition (25). Kosaric & Sukan showed that growth conditions and environmental factors influence on BS<sub>s</sub> production because of their effects on cellular growth and cell activity (26). Type of carbon source influence the final yield of BS. Four potential pathways for BS production: The first one is the hydrophilic and hydrophobic moieties are formed by distinct metabolic pathways. Second, hydrophilic and hydrophobic sides of BS are effected by carbon source. The third theory, the hydrophilic moiety is originated de novo, whereas the carbon source is responsible for hydrophobic side's formation. Finally, the hydrophobic moiety is initiated de novo, whereas the other end is effected by the carbon source (27). Sunflower oil could be used to produce BS because it comprises of a combination of monounsaturated and polyunsaturated fatty acids. The high production of BS<sub>s</sub> when we used sunflower oil may be due to the high content of linoleic acid and used as a carbon source, so the bacteria produce biosurfactant to emulsify the oil and make it more accessible for cellular uptake (28). Other study used different oils such as diesel and kerosene as carbon sources (29). Yeast extract (5 gm/l) also added to the liquid media to improve the bacterial growth and enhanced BS production. When a hydrocarbon source is used as a carbon source, the cellular metabolic is engaged to

the lipolytic pathway and gluconeogenesis, permitting it to produce fatty acids or sugars (7). In the absence of exogenous amino acids, *L.acidophilus* can't grow (30). Complex growth media contain components such as yeast extract, peptone and meat extract could improve probiotic growth. DeMan *et al.*, displayed that yeast extract is very important factor for Lactic acid bacteria growth (31). The substrates used in culture not only effect on BS amounts but also effect on the composition and properties of the products (32).

Emulsification assay are the most efficient method to confirm BS production (33). The capability of BS to emulsify liquids is very important properties. The good emulsification activity is critical for BS to be useful in medical, industrial and environmental uses (34). Oil spreading method was done to confirm the presence of BS<sub>s</sub>, it detects the efficacy of strain for BS production, formation of a clear zone due to diffuse of BS drop on the surface of petri dish contain oil-water interface and reduce the surface tension.

Furthermore bacterial supernatant was extracted using equal amounts of chloroform: methanol (2:1) as mentioned by (17) to obtain partially purified BS<sub>s</sub>. Different solvents system was used in other study like chloroform: methanol (2:1), ethanol and acetone, but the results showed higher yield of BS when they used chloroform: methanol 2:1 (35). Silica gel column (3.5 × 30 cm) was loading by crude BS which liquefied by chloroform. Several researchers were used this technique to purify BS (36).

Also FTIR analysis showed that the observed peak at about 3410 cm<sup>-1</sup> is due to the stretching vibration of the hydroxyl group this might be attributable to the polysaccharide O-H groups. Our result was similar to (36) who confirmed the existence of a largely stretching peak around 3440 cm<sup>-1</sup>. The peak at 2931.8 cm<sup>-1</sup> represented compound have C-H bond indicating the aliphatic chains of lipid. The present results similar to Sharma (13) who showed the C-H stretching vibratins in range 2932 cm<sup>-1</sup>. The peak near 1641 cm<sup>-1</sup> is the absorption peak of the C=N stretching vibration (imine-oxime). The absorption peaks around 1535.3 cm<sup>-1</sup> is the distinctive absorption peak of strong N-O stretching vibration of nitro-compound. The band at 1452.4 cm<sup>-1</sup> is corresponding to the medium C-H bending of alkane. The peaks at 1334.74 cm<sup>-1</sup> was assigning to medium OH bending of carboxylic acid. The band at 1247.94 cm<sup>-1</sup> correspond to the strong CO stretching aromatic ester. The other peaks at 1080.14 and 1022.27 cm<sup>-1</sup> are due to the strong C=O stretching vibration of alkyl groups. The peak around 758.02-

702.09  $\text{cm}^{-1}$  is assigned to the strong C-H bending. According to these findings, there are lipids, sugars, and peptides, the molecular composition of BSs is glycolipoproteins. These observations propose that our substances composed of glycolipoproteins. Hydrophobic part of BSs is comprised of lipid and hydrophilic chain is chiefly composed of sugar. Another study by (37) found that BSs derived from lactic acid bacteria is glycolipoproteins. Also glycolipoprotein was produced by *L. plantarum* SN35N (38). In numerous studies, *Lactobacillus* has been described to produce diverse types of BSs with different chemical natures like glycoproteins, polypeptides, exopolysaccharides, glycolipids and fatty acids which might be due to a diversity of metabolic and genetic variables (39).

Analysis with Gas chromatography–mass spectrometry (GC-MS) of our study was similar to another study which reported that most common fatty acid in *L. plantarum* was hexadecanoic acid, octadecadienoic acid, methyl stearate and methyl ester (40). Ek and Mathew found different lipids in BS produced by *Burkholderia* sp. Includes 9-Octadecenoic acid, Heptadecenoic acid, Hexadecenoic acid, etc (41).

MIC is the lowest concentration of antibiotics that inhibit microorganism growth. Determination of MIC are essential to assess the potency of the antimicrobial agents. Our results exhibited that MIC values of BS against *S. aureus* are 0.625 mg/ml. In previous study, lipopeptide BS give antimicrobial effect against *S. aureus* isolates at MIC 0.500  $\mu\text{g}/\text{mL}$ . Higher value of MIC (25 to 50 mg/ml) of BSs produced from *L. paracasei* was obtained against pathogenic bacteria (42). In addition, the crude BS isolated from *Lactobacillus* exhibited antimicrobial effect toward *S. aureus* and *E. coli* at MIC concentrations ranging between 25 and 50 mg/ml (43).

Biofilm is the significant virulence factors in bacteria, it plays vital role in increase the multi-drug resistant phenomenon in bacteria worldwide (44). Fighting biofilm is an extremely required process (45). Al-Mtory (46) revealed that 100% of *S. aureus* isolates were biofilm producer. On Congo red media, the appearance of black color appeared the capacity of the dye to stain the polysaccharide layer that made through biofilm production (47). Nevertheless, protection mechanisms of biofilm for bacterial population seem to be altered from those responsible for antibiotics resistance (48). The extracellular polymeric substances matrix stops antibiotic action, either by restrictive the diffusion of drug or by

chemical interaction with proteins and extracellular matrix (49). The biofilm was inhibited by biosurfactants and this was indicated by the lack of the dry crystalline black colonies. Recently, BSs have more attention due to their eco-friendly nature, high selectivity and low toxicity (50). It also has efficacy in combat pathogenic bacteria and their biofilms (51). BSs produced a film that modifies the surface wettability and changes the adhesion capability of pathogenic bacteria (52). It has large hampering for the adhesion ability and inhibiting the preformed biofilms of pathogenic bacteria and has effect on the bacterial–surface interface (53). Other research using scanning electron microscope to study the effect of BS produce from *Pediococcus pentosaceus* on bacterial biofilm, they found that BSs give an anti-adhesion ability by its effect on the architecture of the biofilm and decreasing the total extracellular polymeric substances content, disturbed the cell walls integrity and decrease the thickness of biofilm layer. Moreover, in the presence BSs the bacterial cell incapable to keep their usual shape (54). Biofilm matrix that rich with EPS is very essential for the stability of biofilms (55). Consequently, targeting the composition of EPS finally weakens the matrix of biofilm that make the drug easy to entry directly into the biofilms (56). Previous study found that BS extracted from probiotic bacteria was obstruct the bacterial colonization on the medical device (57). BS produced by *Lactobacillus* sp. resulted in a decrease in bacterial biofilm by 85% at concentration 312.5  $\mu\text{g}/\text{mL}$  (58). Higher concentration (25 mg/ml) of BSs produced by lactic acid bacteria showed anti-adhesive activity against *S. aureus* and, *Streptococcus agalactiae* and *S. epidermidis* (59). Van der Mei *et al* found that BSs extracted from *L. fermentum* exhibited anti-adhesive ability against uropathogen (60).

The fibrinogen-binding protein (fib) (clumping factor) is a main virulence factor in *S. aureus*, it has a chief role in biofilm production. The results of molecular docking showed that the active site of fib protein included specific amino acids, number and type of these amino acids different among the ligands. The knowledge of the key amino acids complicated in the interaction have benefit in the design of drugs. There are some non-covalent interactions between the receptor and ligand which are essential in the binding affinity in molecular docking. These include: Hydrogen bonds: these are stronger interaction between a hydrogen atom in ligand and an electronegative atom of the receptor. This bonds are chief to stabilize the complex and aid meaningfully to the binding affinity. Van der Waals interactions:

weak, attractive forces between atoms or molecules. Van der Waals interactions have vital role in the binding affinity. Alkyl: these interactions take place between alkyl groups (carbon chain). Pi-alkyl: these interactions take place between an aromatic ring (ex; benzene ring) and an alkyl groups. The strength of these interactions determine the overall binding affinity between the receptor and ligand. Precisely expecting these interactions is important for molecular docking studies. The active site of the receptor has many amino acids, the position of these amino acids have a large effect on the affinity between the ligand and the receptor (61). These amino acid in the active site of receptor can effect on the binding affinity by influence on the strength of the non-covalent interactions with the ligand. For instance, a mutation of these amino acid can alter the size, shape, or electrostatic features of the binding pocket, which in turn effect on the binding affinity of the ligand. Furthermore, the orientation of amino acids in the active site may effect on the affinity by controlling the convenience and nearness of the ligand to the functional groups in the receptor position (61). In molecular docking, when the value of RMSD is 0.0, this point out that the predicted binding posture of the ligand is matching to the experimental structure of the protein-ligand complex. This refers that the expected structure is an exact equal to the experimental structure, proposing that the docking method has precisely predicted the binding mode of the ligand (62).

RT-qPCR were performed to assess if the anti-biofilm activity of subinhibitory concentrations of BS<sub>s</sub> was reduced gene expression. Housekeeping gene was evaluating the correctness of target gene (63). Other study found relative changes in the expression of biofilm genes and gene involved in quorum-sensing after treatment of *S.aureus* isolates with BS<sub>s</sub> extracted from *Pseudomonas aeruginosa* (64). Liu *et al*, (65) found that surfactant produced from *Bacillus subtilis* was significantly down regulated the expression of polysaccharide and biofilm genes of *Aureus*, which are important to staphylococcal biofilm shape.

### Conclusion

This study revealed that *L. acidophilus*- derived BS displayed a potent inhibition of biofilm production by MDR *S. aureus*, which are prominent biofilm formers on wounds, medical implants and industrial surfaces. This finding indicate that *L. acidophilus* BS can potentially be used as a potent antibacterial and antibiofilm compound, as an alternative to antibiotics or other chemically synthesized toxic agents.

### Acknowledgement

I want to express my gratitude to all those who assisted me throughout this project. I want to express my gratitude to all those who aided me in meeting the criteria for this research.

### Conflict of interest

No conflict of interest is present in the current study.

### Funding

No specific funding source is present but only self-funding by the authors.

### References

- 1- Rayeni LT, Nezhad SS . Characterization of biosurfactant produced by probiotic bacteria isolated from human breast milk. International Journal of Basic Science in Medicine . 2018; 3(1), 18-24. doi 10.15171/ijbsm.2018.04
- 2- Rossi F, Amadoro C, Colavita G . Members of the Lactobacillus genus complex (LGC) as opportunistic pathogens: a review. Microorganisms .2019;7(5), 126. doi:10.3390/microorganisms7050126
- 3- Khamees, L. H., Algburi, A. R., & Mohammed, Z. I. The role of Lactic acid bacteria in combination with conventional antibiotics against bacterial species isolated from genital tract of cattle. . Al-Qadisiyah journal of Veterinary Medicine Sciences. 2022; 21 (2).
- 4- Al-Ali SA., AL-Tae SK , Al-Sabaawy HB. Effect of antibiotic substitution with *Saccharomyces cerevisiae* and probiotic on hematic parameters and growth performance of broilers. Iraqi J Vet Sci .2023; 37(3), 667-673. DOI: 10.33899/ijvs.2023.137187.2648
- 5- Kamaruddin A, Nurhudah M, Rukmono D, Wiradana A, Potential of probiotics *Bacillus subtilis* to reduce ammonia levels, *Vibrio* sp abundance, and increased production performance of Seaworm (*Nereis* sp) under laboratory scale. Iraqi J Vet Sci.2021; 35(4), 757-763. DOI: 10.33899/ijvs.2021.128408.1572
- 6- Farias CB, Almeida FC, Silva IA, Souza TC, Meira HM , Rita E , Sarubbo LA. Production of green surfactants: Market prospects. Electronic Journal of Biotechnology.2021;51, 28-39.. <https://doi.org/10.1016/j.ejbt.2021.02.002>
- 7- Santos DK, Rufino RD, Luna JM , Santos VA, Sarubbo LA. Biosurfactants: multifunctional biomolecules of the 21st century. *Int. J. Mol. Sci.*.2016; 17(3), 401. <https://doi.org/10.3390/ijms17030401>
- 8- Jahan R, Bodratti AM, Tsiano M , Alexandridis P. Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and



- applications. *Adv. Colloid Interface Sci.* . 2020; 275, 102061. <https://doi.org/10.1016/j.cis.2019.102061>
- 9- Khadim, R. N., & Al-Husseiny, S. H. Molecular Detection of Staphylococcus spp in Ovine Actinomycosis. *Al-Qadisiyah journal of Veterinary Medicine Sciences.* 2024; 23 (1).
- 10- Liu HY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. *npj Antimicrob Resist.* 2024; 2, 27 . <https://doi.org/10.1038/s44259-024-00046-3>
- 11-Murray CJ, Ikuta KS , Sharara F, Swetschinski L, Aguilar GR ,Gray A , Tasak N. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet.* 2022; 399(10325), 629-655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- 12- Tekleyohannes KM. Screening and characterization of a biosurfactant producing bacterium isolated from ethiopian soda lake and assessment of its compatibility in a laundry detergent (doctoral dissertation, addis ababa science and technology university). 2021; doi .10.20372/nadre:2876
- 13- Sharma A, Soni J , Kaur G , Kaur J. A study on biosurfactant production in Lactobacillus and Bacillus sp. *Int. J. Curr. Microbiol.*2014; *App. Sci.* 3(11), 723-733. <https://www.ijcmas.com/vol-3-11/Anayata%20Sharma,%20et%20al.pdf>
- 14- Barakat KM, Hassan SW , Darwesh OM . Biosurfactant production by haloalkaliphilic Bacillus strains isolated from Red Sea, Egypt. *Egypt J Aquat.*2017; *Res* 43(3):205–211. <https://doi.org/10.1016/j.ejar.2017.09.001>
- 15- Morikawa M, Hirata Y, Imanaka T. A study on the structure-function relationship of lipopeptide biosurfactants. *Bioch. et Bioph. Acta (BBA) - Molecular and Cell Biology of Lipids* .2000; 1488, (3) 211-218. [https://doi.org/10.1016/S1388-1981\(00\)00124-4](https://doi.org/10.1016/S1388-1981(00)00124-4)
- 16- El-Sheshtawy HS , Aiad I , Osman ME, Abo-ELnasr AA, Kobisy AS. Production of biosurfactant from Bacillus licheniformis for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria. *Egyptian Journal of Petroleum.* 2015;24(2), 155-162. <https://doi.org/10.1016/j.ejpe.2015.05.005>
- 17- Darvishi P, Ayatollahi S, Mowla D, Niazi, A. Biosurfactant production under extreme environmental conditions by an efficient microbial consortium, ERCPP1-2. *Colloids and Surfaces B: Biointerfaces.*2011; 84(2), 292-300. <https://doi.org/10.1016/j.colsurfb.2011.01.011>
- 18- Sánchez M, Teruel JA , Espuny MJ , Marqués A, Aranda FJ , Manresa A , Ortiz A. Modulation of the physical properties of dielaidoylphosphatidylethanolamine membranes by a dirhamnolipid biosurfactant produced by Pseudomonas aeruginosa. *Chemistry and physics of lipids.* 2006; 142(1-2), 118-127. <https://doi.org/10.1016/j.chemphyslip.2006.04.001>
- 19- Ragavendran P , Sophia D, Arul Raj C, Gopalakrishnan VK. Functional group analysis of various extracts of Aerva lanata (L.,) by FTIR spectrum. *Pharmacologyonline.* 2011; 1, 358-364. <https://www.researchgate.net/publication/215547192>
- 20- Zheng C, He J. Wang Y. Wang M. Huang Z . Hydrocarbon degradation and bioemulsifier production by thermophilic Geobacillus pallidus strains. *Bioresource technology* .2011; 102(19), 9155-9161. <https://doi.org/10.1016/j.biortech.2011.06.074>
- 21- Clinical Laboratory Standards Institute CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 9th ed., CLSI document M07-A9.* Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.2012.
- 22- Melo PD, Ferreira LM , Nader Filho A, Zafalon LF , Vicente HI, SouzaVD. Comparison of methods for the detection of biofilm formation by Staphylococcus aureus isolated from bovine subclinical mastitis. *Brazilian Journal of Microbiology.* 2013; 44, 119-124. <https://doi.org/10.1590/S1517-83822013005000031>
- 23- Didierjean C, Tête-Favier F Introduction to Protein Science. *Architecture, Function and Genomics.* By Arthur M. Lesk. Oxford University Press, 2016. Pp. 466. Paperback. Price GBP 39.99. ISBN 9780198716846. *Acta Crystallographica Section D: Structural Biology.* 2016; 72(12), 1308-1309. <https://doi.org/10.1107/S2059798316018283>
- 24- Dukhande M, Warde M. Isolation and characterization of potent biosurfactant producing bacteria from petroleum contaminated soil and sea water. *Int J Eng Res Technol.* 2016; 5(3). DOI : 10.17577/IJERTV5IS030332
- 25- Saeed ZK , Abbas BA , Othman RM. Molecular identification and phylogenetic analysis of lactic acid bacteria isolated from goat raw milk. *Iraqi J Vet Sci.* 2020; 34(2), 259-263. DOI:10.33899/ijvs.2019.125896.1176
- 26-Kosaric N, Sukan FV. Biosurfactants: production: properties: applications. CRC Press. 504pp. 2010; <https://doi.org/10.1201/9780585355702>



- 27- Nurfarahin AH, Mohamed MS, Phang LY. Culture medium development for microbial-derived surfactants production—an overview. *Molecules*. 2018; 23(5), 1049. <https://doi.org/10.3390/molecules23051049>
- 28- Wasoh H, Baharun S, Halim M, Lajis AF, Ariff A, Lai OM. Production of rhamnolipids by locally isolated *Pseudomonas aeruginosa* using sunflower oil as carbon source. *Bioremediation Science and Technology Research*. 2017; 5(1), 1-6. <https://doi.org/10.54987/bstr.v5i1.350>
- 29- Mnif I, Ellouze-Chaabouni S, Ayedi Y, Ghribi D. Treatment of diesel-and kerosene-contaminated water by *B. subtilis* SPB1 biosurfactant-producing strain. *Water Environment Research*. 2014; 86(8), 707-716. <https://doi.org/10.54987/bstr.v5i1.350>
- 30- Morishita T, Deguchi Y, Yajima M, Sakurai T, Yura T. Multiple nutritional requirements of lactobacilli: genetic lesions affecting amino acid biosynthetic pathways. *J. Bacteriol.* 1981; 148(1), 64-71. <https://doi.org/10.1128/jb.148.1.64-71.1981>
- 31- de Man JD, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli. *J. Appl. Microbiol.* 1961; 23(1), 130-135. <https://doi.org/10.1111/j.1365-2672.1960.tb00188.x>
- 32- Langer O, Palme O, Wray V, Tokuda H, Lang S. Production and modification of bioactive biosurfactants. *Process Biochemistry*. 2006; 41(10), 2138-2145. <https://doi.org/10.1016/j.procbio.2006.07.036>
- 33- Amaral PF, Da Silva JM, Lehocky BM, Barros-Timmons AM, Coelho MA, Marrucho IM, Coutinho JA. Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. *Process Biochemistry*. 2006; 41(8), 1894-1898. <https://doi.org/10.1016/j.procbio.2006.03.029>
- 34- Al-Seraih AA, Swadi WA, Al-hejjaj MY, Al-Laibai, Ghadban AK. Isolation and Partial Characterization of Glycolipopeptide Biosurfactant Derived from A Novel *Lactiplantibacillus plantarum* Lbp\_WAM. *Basrah Journal of Agricultural Sciences*. 2022; 35(2), 78-98. <https://doi.org/10.37077/25200860.2022.35.2.06>
- 35- Rasheed HG, Haydar NH. Purification, characterization and evaluation of biological activity of Mannoprotein produced from *Saccharomyces cerevisiae* BY. *Iraqi Journal of Agricultural Sciences*. 2023; 54(2), 347-359. DOI: <https://doi.org/10.36103/ijas.v54i2.1709>
- 36- Jameel AA, Haider NH. Study the antimicrobial and antiadhesive activity of purified biosurfactant produced from *Lactobacillus plantarum* against pathogenic bacteria. *Iraqi Journal of Agricultural Sciences*. 2021; 52(5), 1194-1206. <https://doi.org/10.36103/ijas.v52i5.1457>
- 37- Nataraj BH, Ramesh C, Mallappa RH. Characterization of biosurfactants derived from probiotic lactic acid bacteria against methicillin-resistant and sensitive *Staphylococcus aureus* isolates. *Lwt*. 2021; 151, 112195. <https://doi.org/10.1016/j.lwt.2021.112195>
- 38- Lara VM, Vallejo M, Parada R, Henao Ossa JS, Gliemmo MF, Campos CA. Characterization of the emulsifying activity of biosurfactants produced by lactic acid bacteria isolated from the Argentinian Patagonia. *Journal of Dispersion Science and Technology*. 2020; 43(6), 902-909. <https://doi.org/10.1080/01932691.2020.1845961>
- 39- Vallejo CM, Restrepo MA, Duque FL, Díaz, JC. Production, characterization and kinetic model of biosurfactant produced by lactic acid bacteria. *Electronic Journal of Biotechnology*. 2021; 53, 14-22. <https://doi.org/10.1016/j.ejbt.2021.06.001>
- 40- Thakur B, Kaur S, Dwivedi V, Albadrani GM, Al-Ghadi MQ, Abdel-Daim MM. Unveiling the antimicrobial and antibiofilm potential of biosurfactant produced by newly isolated *Lactiplantibacillus plantarum* strain 1625. *Frontiers in Microbiology*. 2024; 15, 1459388. <https://doi.org/10.3389/fmicb.2024.1459388>
- 41- EK R, Mathew J. Characterization of biosurfactant produced by the endophyte *Burkholderia* sp. WYAT7 and evaluation of its antibacterial and antibiofilm potentials. *J. Biotechnol.* 2020; 313, 1-10. <https://doi.org/10.1016/j.jbiotec.2020.03.005>
- 42- Sharma D, Saharan BS. Functional characterization of biomedical potential of biosurfactant produced by *Lactobacillus helveticus*. *Biotechnology Reports*. 2016; 11, 27-35. <https://doi.org/10.1016/j.btre.2016.05.001>
- 43- Sambanthamoorthy K, Feng X, Patel R, Patel S, Paranavitana C. Antimicrobial and antibiofilm potential of biosurfactants isolated from lactobacilli against multi-drug-resistant pathogens. *BMC microbiology* 2014; 14, 1-9. <https://doi.org/10.1186/1471-2180-14-197>
- 44- Ramachandran G, Alharbi NS, Chackaravarthy G, Chelliah CK, Rajivgandhi G, Maruthupandy M, Li WJ. Chitosan/silver nanocomposites enhanced the biofilm eradication in biofilm forming Gram positive *S. aureus*. *Journal of King Saud University-Science*. 2023; 35(4), 102597. <https://doi.org/10.1016/j.jksus.2023.102597>



- 45- Neamah AJ , Al-Al-Yassari AK , Hamed MA, AlRammahi, MA. Inhibition of Escherichia coli biofilm formation by Streptomyces sdLi crude extract. Iraqi J Vet Sci .2020; 34(2), 305-310. DOI: 10.33899/ijvs.2019.125965.1202,
- 46- Al-Mtory HK. Bacteriological and Molecular Study of Staphylococcus spp. Isolated from Clinical Specimen. MSc thesis. College of medicine Babylon University. 2016.
- 47- Bose S,Khodke M , Basak S, Mallick SK. Detection of biofilm producing staphylococci: need of the hour. Journal of clinical and diagnostic research .2009; 3(6), 1915-1920. DOI: <https://doi.org/10.7860/JCDR/2009/600>
- 48- Glinel K , Thebault P, Humblot V, Pradier CM, Jouenne T. Antibacterial surfaces developed from bio-inspired approaches. Acta biomaterialia.2012; 8(5), 1670-1684. <https://doi.org/10.1016/j.actbio.2012.01.011>
- 49- Simões M, Simões LC, Vieira MJ. A review of current and emergent biofilm control strategies. LWT-Food Science and Technology.2010; 43(4), 573-583. <https://doi.org/10.1016/j.lwt.2009.12.008>
- 50- Patel K, Patel,M. Improving bioremediation process of petroleum wastewater using biosurfactants producing Stenotrophomonas sp. S1VKR-26 and assessment of phytotoxicity. Bioresource technology.2020; 315, 123861. <https://doi.org/10.1016/j.biortech.2020.123861>
- 51- Singh P, Cameotra SS. Potential applications of microbial surfactants in biomedical sciences. TRENDS in Biotechnology. 2004; 22(3), 142-146. doi: 10.1016/j.tibtech.2004.01.010. PMID: 15036865.
- 52- Arutchelvi JI, Bhaduri S, Uppara PV, Doble M. Mannosylerythritol lipids: a review. Journal of Industrial Microbiology and Biotechnology.2008 ; 35(12), 1559-1570. <https://doi.org/10.1007/s10295-008-0460-4>
- 53- Banat IM, De Rienzo MA, Quinn GA. Microbial biofilms: biosurfactants as antibiofilm agents. *Appl. Microbiol. Biotechnol.*. 2014 ; 98, 9915-9929. <https://doi.org/10.1007/s00253-014-6169-6>
- 54- Adnan M , Siddiqui AJ , Hamadou WS, Ashraf SA , Hassan MI , Snoussi M, Patel, M. Functional and structural characterization of pediococcus pentosaceus-derived biosurfactant and its biomedical potential against bacterial adhesion, quorum sensing, and biofilm formation. Antibiotics. 2021; 10(11), 1371. <https://doi.org/10.3390/antibiotics10111371>.
- 55- Kim D , Hwang G, Liu Y, Wang Y , Singh AP , Vorsa N, Koo H. Cranberry flavonoids modulate cariogenic properties of mixed-species biofilm through exopolysaccharides-matrix disruption. PLoS One. 2015; 10(12), e0145844. <https://doi.org/10.1371/journal.pone.0145844>
- 56- Lentino JR. Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. *Clin. Infect. Dis.*. 2003; 36(9), 1157-1161. <https://doi.org/10.1086/374554>
- 57- Falagas ME , Makris GC. Probiotic bacteria and biosurfactants for nosocomial infection control: a hypothesis. J. Hosp. Infect. . 2009; 71(4), 301-306. <https://doi.org/10.1016/j.jhin.2008.12.008>
- 58- Fracchia L, Cavallo M, Allegrone G, Martinotti MG. A Lactobacillus-derived biosurfactant inhibits biofilm formation of human pathogenic Candida albicans biofilm producers. Appl Microbiol Biotechnol.2010; 2, 827-837.
- 59- Gudiña EJ, Rocha V, Teixeira JA, Rodrigues LR. Antimicrobial and antiadhesive properties of a biosurfactant isolated from Lactobacillus paracasei ssp. paracasei A20. Letters in applied microbiology.2010; 50(4), 419-424. <https://doi.org/10.1111/j.1472-765X.2010.02818.x>
- 60- Van der Mei HC , Free RH, Elving GJ, Van Weissenbruch R, Albers FW, Busscher HJ. Effect of probiotic bacteria on prevalence of yeasts in oropharyngeal biofilms on silicone rubber voice prostheses in vitro. *J. Med. Microbiol.* . 2000; 49(8), 713-718. <https://doi.org/10.1099/0022-1317-49-8-713>
- 61- Maden SF, Sezer S, Acuner SE. Fundamentals of molecular docking and comparative analysis of protein–small-molecule docking approaches. In Molecular Docking-Recent Advances. IntechOpen. 2022; DOI: 10.5772/intechopen.105815
- 62- Mandal SK , Munshi P. Predicting Accurate Lead Structures for Screening Molecular Libraries: A Quantum Crystallographic Approach. Molecules. 2021; 26(9), 2605. <https://doi.org/10.3390/molecules26092605>
- 63- Li HB, Dai CG , Zhang CR , He YF, Ran HY, Chen SH. Screening potential reference genes for quantitative real-time PCR analysis in the oriental armyworm, Mythimna separata. PLoS One. 2018; 13(4), e0195096. <https://doi.org/10.1371/journal.pone.0195096>
- 64- Saadati F, Shahryari S, Sani NM, Farajzadeh D, Zahiri HS, Vali H, Noghabi KA. Effect of MA01 rhamnolipid on cell viability and expression of quorum-sensing (QS) genes involved in biofilm formation by methicillin-resistant Staphylococcus aureus. Scientific Reports.2022; 12(1), 14833. <https://doi.org/10.1038/s41598-022-19103-w>
- 65- Liu J, Li W, Zhu X, Zhao H, Lu Y, Zhang C, Lu Z. Surfactin effectively inhibits Staphylococcus aureus adhesion and biofilm formation on surfaces.



Applied microbiology and 33- Kosaric, N., & Sukan, F. V. (2010). Biosurfactants: production: properties: applications. CRC Press.2019; 504pp. doi: 10.1007/s00253-019-09808-w.