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

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Abstract Lactic acid bacteria are a member of probiotic bacteria and their metabolites such as biosurfactants (BSs) emerged as great alternative for antibiotics due to their activities such as antimicrobial, antiadhesive and immune-modulatory effects. The current study aimed to produce, purify and evaluate the antibiofilm effect of BSs produced by *Lactobacillus acidophilus* isolate against multidrug resistant (MDR) *Staphylococcus aureus*. BSs were screened by preliminary tests like emulsification assays , oil spreading test and foaming activity. Purification of BSs was done by silica column chromatography and then analyzed by FTIR and Gas chromatography–mass spectrometry. Broth macro-dilution method was employed to find Minimum inhibitory concentration (MIC) of BSs. Quantitative Reverse Transcriptase Real-Time PCR was done to study the gene 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 BSs**.** Emulsification assay for BSs were 67% , clear zone (diameter 7 cm) was formed on the surface of petri dish contain oil-water interface. The foaming activity displayed the percentage of 60%. FTIR analysis proposed that it composed of glycolipoproteins. GC-MASS showed the presence of different biologically compounds such as Benzoic acid , n-Hexadecanoic acid , Linoelaidic acid. The MIC values of BSs 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 were produced biofilm after treated with sub-MIC of BSs. The chemical compounds in BSs 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). Expression level of *fib* gene was significantly decreased at concentrations 320 µg/mL. In conclusion, this study provides an effective method to inhibit the biofilm by MDR *S. aureus.*

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

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). BSs is a chemical substances produced from LAB. It is amphipathic molecules with hydrophilic and hydrophobic moieties (6). BSs 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, BSs 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

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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 BSs 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 37^0C 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 25^0C overnight. The emulsification index (E24) estimated according to the following equation (14).

E24 = Total height of emulsion / 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 shaked by hand for two minutes. The foam activity was measured by the following equation (16).

Foaming = foam height / 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 a incessant flow of methanol then wash the column by methanol. 10 ml of methanol was used to dissolve BSs (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 BSs experienced for extra tests (18).

Characterization of purified biosurfactant

FTIR analysis of Biosurfactants

The functional groups of BSs 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

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mg) at 90 ^{0}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 BSs was measured by the modified broth macro-dilution method. A twofold serial dilution of BSs was prepared by dissolving the dried BSs 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 ×10^8 cell/ml). The tubes incubated at at 37 ^{o}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 121^0 C for 15 minutes, then the solution was mixed with brain heart infusion agar at 55^0 C. The sugar was mixed with other components. Antibiofilm activity of BSs were tested by growing the isolates with and without sub-MIC of BSs and then cultured on CRA plates. Plates incubated aerobically at 37^0 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 BSs 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 were 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 (RSMD) 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 *rpo*B .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

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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:ACATTCGAATATGGTGCACGTC	142 bp	PP537160.1
	R:TTCTGTGTGCACTGACAGTATG		
rpoB gene	F:CGTGCTCAAATGGAAGTACGTG	77 bp	KU555590.1
	R:GGTCCCTCAGGTGTTTCAATTG		

Results

Lyophilized isolate were culture in MRS agar as shown in figure (1). The isolate were 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 were 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

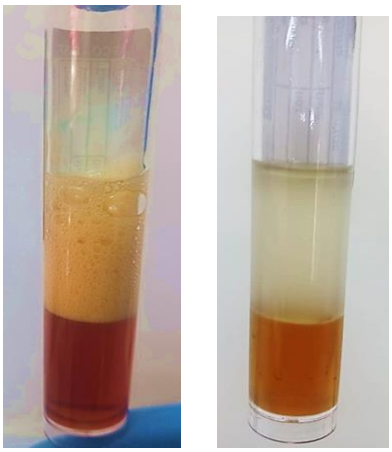

Figure 2: Emulsification assay and Foaming activity of *L. acidophilus* isolate **A:-Emulsificationassays B:- Foaming activity**

Also the foaming activity of *L.acidophilus* isolate was found 60% (figure 2-B). Foaming production is an important test to examine BS_S 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_S . BS_S 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.

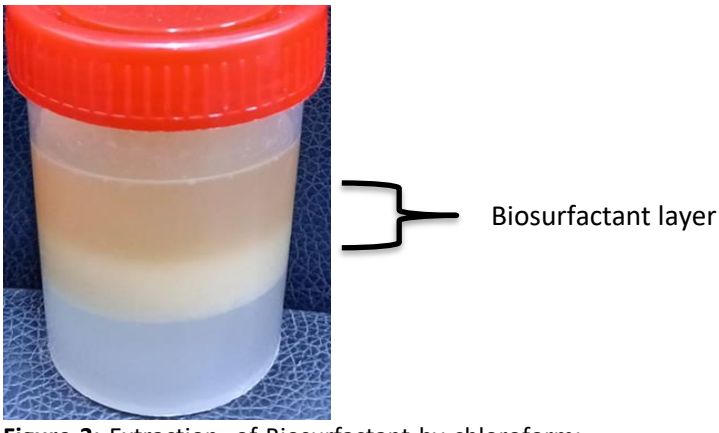

Figure 3: Extraction of Biosurfactant by chloroform: methanol

Silica column chromatography was used in this study to purify BS_S 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 ,

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the surface tension and emulsification activity for any fraction tested.

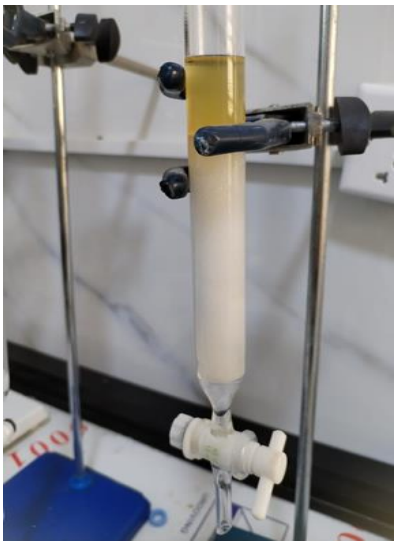

Figure 4: Extraction of Biosurfactant by silica column chromatography technique

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 cm^{-1} region (Figure 5) . FTIR analysis showed that the observed peak at about 3410 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 cm−1 represented compound have C-H bond indicating the aliphatic chains of lipid .The peak near 1641 cm−1 is the absorption peak of the C=N stretching vibration (imine-oxime). The absorption peaks around 1535.3 cm−1 is the distinctive absorption peak of strong N-O stretching vibration of nitro-compound. The band at 1452.4 cm−1 is corresponding to the medium C-H bending of alkane. The peaks at 1334.74 cm−1was assigning to medium OH bending of carboxylic acid. The band at 1247.94 cm−1 correspond to the strong CO stretching aromatic ester. The other peaks at 1080.14 and 1022.27 cm−1 are due to the strong C=O stretching vibration of alkyl groups. The peak around 758.02–702.09 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_S is glycolipopeptides .These observations propose that our substances composed of glycolipoproteins. Hydrophobic part of BS_S is comprised of lipid and hydrophilic chain is chiefly composed of sugar.

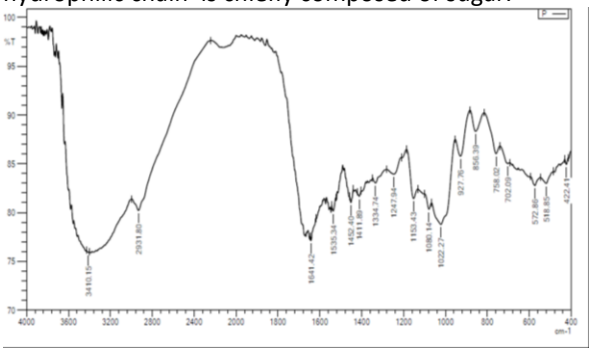

Figure 5: FTIR spectrum analysis of biosurfactant produced from *L. acidophilus*

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 acidmonounsaturated fatty acid and unsaturated fatty aldehyde as shown in Table (2) .

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

Number of peaks	Retention time (minute)	Compound	Group	area	Area %
1	49.987	Benzoic acid, phenylmethyl ester	aromatic ester	3472540	1.26
2	56.491	n-Hexadecanoic acid	carboxylic acids(saturated fatty acid)	60042039	21.73

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3	56.771	n-Hexadecanoic acid	carboxylic acids(saturated fatty acid)	729508	0.26
4	61.863	Linoelaidic acid	polyunsaturated fatty acid	35939638	13.01
5	62.063	9-Octadecenoic acid	monounsaturated fatty acid	100673743	36.44
6	62.200	9-Octadecenoic acid	monounsaturated fatty acid	49197835	17.81
7	62.721	1Heptadecanecarboxylic acid	saturated fatty acid	17802122	6.44
8	64.612	Linoelaidic acid	polyunsaturated fatty acid	46782	0.02
9	66.310	9,12-Octadecadienoic acid	monounsaturated fatty acid	428903	0.16
10	67.847	Cyclopropaneoctanal, 2-octyl-	polyunsaturated fatty acid	7619577	2.76
11	68.007	cis-9-Hexadecenal	unsaturated fatty aldehyde	328915	0.12

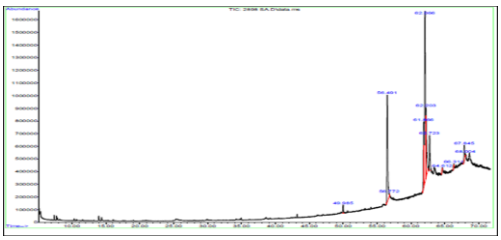

Figure 6: GC analysis of purified biosurfactants produce from *L.acidophilus*

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^2	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	Binding Affinity	rmsd/ub	rmsd/lb
1n67_Fib_protein_clear_Benzoic_acid	-7.5	0	0
1n67_Fib_protein_clear_9	-6.3	0	0

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1n67_Fib_protein_clear_n-Hexadecanoic_acid_uff_E=57.46	-6	0	0
1n67_Fib_protein_clear_Linoelaidic_acid_uff_E=81.58	-6	0	0
1n67_Fib_protein_clear_Cyclopropaneoctanal	-6	0	0
1n67_Fib_protein_clear_9-Octadecenoic_acid_uff_E=69.17	-5.8	0	0
1n67_Fib_protein_clear_1-Heptadecanecarboxylic_acid_uff_E=41.91	-5.5	0	0
1n67_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.

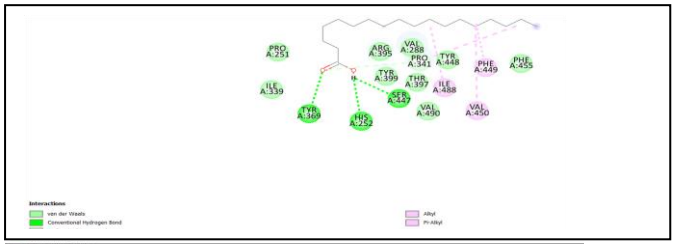

Figure 7: Benzoic acid interaction with fib protein of *S. aureus*

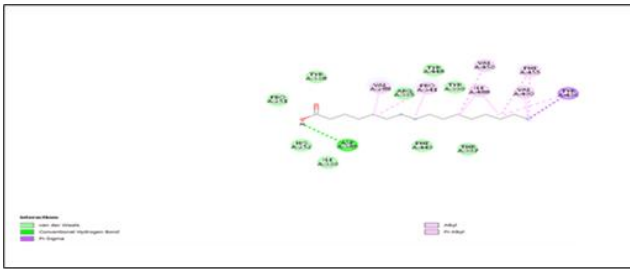

Figure (8): n-Hexadecanoic acid interaction with fib protein of *S. aureus*

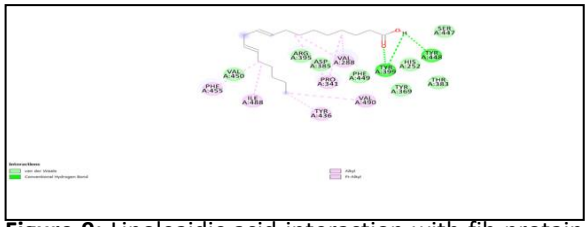

Figure 9: Linoleaidic acid interaction with fib protain of *S. aureus*

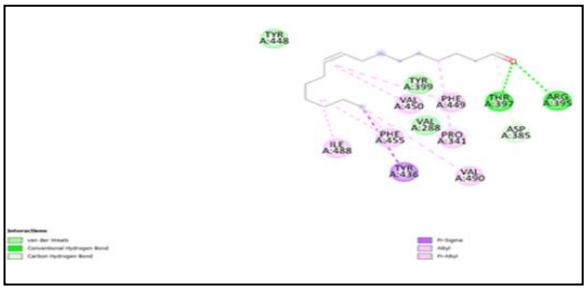

Figure 10: Cyclopropaneoctanal interaction with fib protein of *S. aureus*

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

The *fib* gene expression were assessed before and after treatment of *S. aureus* isolates with three concentrations of sub –MIC of BS_S (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_S 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

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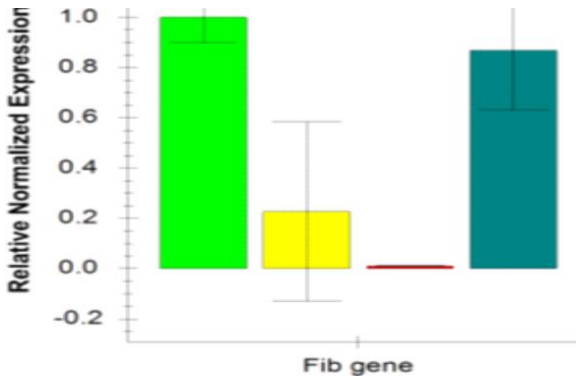

Figure 11: The effect of Biosurfactants produce from *L.acidophilus* on the expression of *fib* gene of *S.aureus*

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

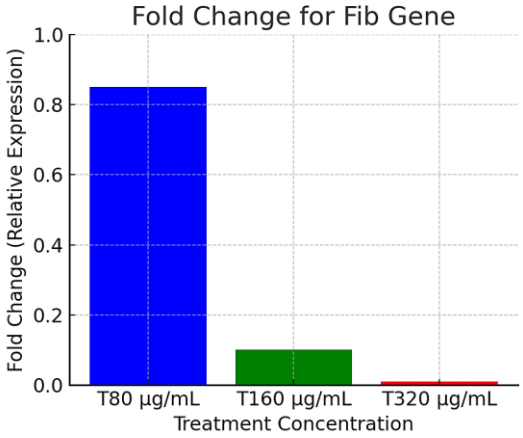

Figure 12: Fold change analysis for the *fib* gene of *S. aureus* treated with different concentrations of Biosurfactants

Discussion

Culture conditions of *L.acidophilus* were optimized to produce BSs 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 BSs 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 BSs 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 BSs , it detect 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 BSs . Different solvents system were 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−1 is due to the stretching vibration of

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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^{-1} . The peak at 2931.8 cm−1 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 in range 2932 cm−1. The peak near 1641 cm−1 is the absorption peak of the C=N stretching vibration (imine-oxime). The absorption peaks around 1535.3 cm−1 is the distinctive absorption peak of strong N-O stretching vibration of nitro-compound. The band at 1452.4 cm−1 is corresponding to the medium C-H bending of alkane. The peaks at 1334.74 cm−1was assigning to medium OH bending of carboxylic acid. The band at 1247.94 cm−1 correspond to the strong CO stretching aromatic ester. The other peaks at 1080.14 and 1022.27 cm−1 are due to the strong C=O stretching vibration of alkyl groups. The peak around 758.02–702.09 cm−1 is assigned to the strong C-H bending. According to these finding, there are lipid, sugars, and peptides , the molecular composition of BSs is glycolipopeptides .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 glycolipopeptide 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 preveous study, lipopeptide BS give antimicrobial effect against *S. aureus* isolates at MIC 0.500 µg/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

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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 µg/mL (58). Higher concentration (25 mg/mL) of BS_S 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 BS_S 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 have 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 is 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 interaction take place between alkyl groups (carbon chain). Pi-alkyl : these interaction 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 have 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_S was reduced gene expression. Housekeeping gene was evaluate 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_S 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 *S.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.

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Conflict of interest

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