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Antibacterial Activity of Biosurfactant extracted from *Streptococcus thermophilus* and Its effect on some Biochemical Parameters in Male Rats

Batool Shakir Abed Almjalawi[1*], Thoalffakar A. Alhamed[2], Ahmed Abdulkareem Almutteari [3]

1. General Directorate of Education in Karbala, Karbala, Iraq
2. University of Warith AL-Anbiyaa, College of Nursing, Karbala, Iraq
3. General Directorate of Education in Karbala, Karbala, Iraq

** Corresponding author's E-mail: batoolshakir5@gmail.com*

ABSTRACT

Streptococcus Thermophilus belongs to the group of lactic acid bacteria. The current study aims to identify the role of the biosurfactant produced by *S. thermophilus* bacteria, showing its importance in limiting the growth of pathogens and its effect on the biochemical parameters of male rats. *S. thermophilus* bacteria were isolated from local cheese, 120 different samples were collected from the human body (urine, sputum, wounds), and the biosurfactant was extracted from the bacteria. the active groups of biosurfactant components were detected and the antibacterial activity was tested on some biochemical parameters in male rats. 42 infected samples were distributed between 16 for urine, 11 for sputum and 15 for wound samples. The results showed that the highest percentage of infection with *Staphylococcus aureus* was 12 (28.57%), while the lowest *Staphylococcus epidermidis* was 3 (7.14%). The qualitative tests showed that the biosurfactant contains many active components such as tannins, carbohydrates, phenols, flavonoids, and sapindales, while does not contain triterpenoids. The results of the statistical analyses to anti-bacterial activity showed a significant increase ($p<0.05$), which indicates its effect on some biochemical parameters in male rats. A significant decrease was found in the total cholesterol, triglycerides and high-density lipoprotein (LDL) concentrations in the treatment group in comparison with the control. It was also noted a significant increase in HDL compared to control group. We conclude that biosurfactant has the ability to eliminate some gram-positive and gram-negative bacteria isolated from different clinical infections of the human body and it has an effective effect on the biochemical parameters of the male rats.

Keywords: Biosurfactant, *Streptococcus thermophilus*, Biochemical parameters, Rats.
Article type: Research Article.

INTRODUCTION

Streptococcus thermophilus is a bacterium that belongs to the group of lactic acid bacteria and it is described as gram-positive, spherical in shape, arranged in pairs or chains, anaerobic. Its optimum temperature for growth is 37 °C and it has the ability to grow at high temperatures (Hardie & Whiley 1995; Robinson *et al.* 2002). The bacterium possesses the characteristics of probiotics, hence is widely used in this field. Historically it has been used safely as a starter in the manufacture of yoghurt (Akpinar *et al.* 2011). Lactic acid bacteria, including *S. thermophilus*, produce many substances that inhibit microbial growth and have inhibitory effects on the growth of pathogenic microbes and those that cause food spoilage. Among those substances are lactic acid, acetic acid, formic acid, ethanol, hydrogen peroxide, diacetyl, bacteriocins, and fatty acids (Šušković *et al.* 2010; Vuyst & Leroy 2010; Ukeyima *et al.* 2010). This starter bacterium is effective in the prevention and treatment of some diseases caused by pathogenic microbes by several mechanisms, including the production of many inhibitory substances and its ability to inhibit the colonization of pathogenic bacteria and treat infections of the digestive

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system, especially those caused by *Clostridium difficile* and *Helicobacter pylori* (Petti *et al.* 2008). It also plays a role in reducing lactose intolerance problems (FAO & WHO 2002). *S. thermophilus* is one of the most important lactic acid bacteria used in fermented dairy starters, as it is used in the manufacture of yoghurt and some types of cheese (Delcour *et al.* 1996; Parente & Cogan 2004), in addition to its role in inhibiting diarrhoea-causing bacteria (Nurhajati *et al.* 2008). *S. thermophilus* or its bacteriocins are used as probiotics and inhibition of damaged bacteria such as *Clostridium sporogenes* and *C. tyrobutyricum*. The bacteriocin produced from these bacteria called Thermophilin has a wide efficacy against many pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* (Hassam & Frank 2001; Aslam *et al.* 2011). *S. thermophilus* also produces biosurfactant and inhibitory activity against bacteria, fungi and viruses (Rodrigues *et al.* 2006). Biosurfactant is known as biological substances that reduces surface tension produced by some microbes and act as anti-microbial and anti-adhesion materials as well as getting rid of some microorganisms such as polysaccharide-protein complex, fatty acids, phospholipids, lip bio-lipid, and glycolipid. It plays a role in inhibiting the adhesion of pathogens that cause urinary tract infections and is also used as a preventive agent to prevent the adhesion of carcinogenic bacteria (Rodrigues *et al.* 2006). Due to the lack of local studies on biosurfactant production of *S. thermophilus* bacteria isolated from Iraqi local cheese and the importance of these therapeutic and preventive bacteria, this study aims to produce a biosurfactant from *S. thermophilus* and its anti-bacterial activity, as well as its effect on some biochemical parameters of male rats.

MATERIALS AND METHODS

1- Bacterial isolates

Streptococcus thermophilus: The bacteria were isolated from local cheese manufactured in the traditional method, *S. thermophilus* were selected from a group of isolates on MRS agar, and their diagnosis was confirmed by following the culture and biochemical tests (Hardie & Whiley 1995).

Pathological bacterial isolates: Pathogenic bacteria were isolated from different clinical infections, 120 different samples of the human body (urine, sputum, wounds) were collected from adult patients of both sexes (males and females). The samples were cultured on a medium of blood agar, then transferred to selective media, Thereafter the isolates were diagnosed on culture, microscopic, and biochemical characteristics (Forbes *et al.* 2002). In addition, the kit APi Staph, APi-20 E and Vitek 2 Compact system were used to confirm the diagnosis.

2- Extraction of Biosurfactant from *S. thermophilus*

Biosurfactant was extracted from *S. thermophilus* according to the methodology of Rodrigues et al. (2006). MRS broth was inoculated with 24-hour-old, *S. thermophilus* bacteria culture; incubated at 37°C for 18 hours; centrifuged at 10,000 rpm for 5 minutes; washed twice; cells were re-suspended with PBS solution and left at room temperature for 2 hours on a magnetic stirrer; centrifuged to remove cell residues; then filtered the liquid through 0.22 µm millipore filters to obtain the biosurfactant. Lyophilizer was used to dry the biosurfactant to obtain a powder, and then it was kept at 4° C until use.

3- Detection of the active groups for biosurfactant

The active groups of biosurfactant which were extracted from *S. thermophilus* isolated from Iraqi local cheese, included tannins (Ahmed *et al.* 1989), carbohydrates (Meyer & Wather 1988), phenols (Adedayo *et al.* 2001), flavonoids (Al-khazraji 1991), fuocuomarins, triterpenoids, and saponins (Harborne 1984).

4- Antibacterial activity of the biosurfactant produced from *S. thermophilus*

The well diffusion method was used to estimate the effectiveness of biosurfactant in inhibiting growth of pathogenic bacteria under examination (Gupta *et al.* 1998).

5-Effect of the biosurfactant produced from *S. thermophilus* on some biochemical parameters in male rate

In the current study, 24 male rats were used, and their weights were ranged between 250 and 300 g. The rats were distributed into two groups and placed in designated cages. All the laboratory conditions were established such as ventilation, lighting and temperature (20-30 °C). The rats were given pellet-type feed that were obtained from specialized animal feed sources.

The animals were randomly divided into two equal groups with 12 rats in each group as the following:

Control group (C): this group was dosed with regular drinking water for the duration of the three week experiment.

Treatment group (T): this group was dosed with biosurfactant solution for the duration of the experiment of three weeks

Samples of collection

Animals were anesthetized with chloroform, and 5-mL blood was taken from the heart by heart puncture after 24 hours from the last dose of the biosurfactant solution. The blood was placed in tubes free of anticoagulant, left for 15-20 minutes at laboratory temperature. The blood serum was separated using a centrifuge at a speed of 3000 rpm for 15 minutes. The serums were kept in a refrigerator at 4°C to measure some of the biochemical parameters.

Biochemical tests

Some Biochemical tests were performed for the male rat blood serum, including total cholesterol (Richmond 1973), Determine triacylglycerol level (Allain et al. 1974), high-density lipoproteins (HDL) (Burstein *et al.* 1970) and low- density lipoproteins (LDL) (Friedewald *et al.* 1972; Chotkowska *et al.* 2001).

Statistical analyses

The statistical analyses on the data were performed using the t-test and one-way analysis of variance according to the computer statistical system (SPSS Ver. 22) and also the standard deviation were assessed as explained by Seltman (2012).

RESULTS AND DISCUSSION

S. thermophilus bacteria were obtained from Iraqi local cheese manufactured in the traditional method and the bacteria were selected from a group of isolates on MRS agar. The diagnosis was confirmed according to the culture and biochemical tests mentioned by Hardie & Whiley (1995).

Isolation and identification of pathogenic bacteria

42 infected samples were obtained, and were distributed between 16, 11 and 15 for urine, sputum and wound samples, respectively. The results of phenotypic, microscopic examinations and biochemical tests showed the infection of Gram-positive and Gram-negative bacteria, and according to the use of API Staph, API 20E, and Vitek 2 Compact System. The results in Table 1 show that the highest percentage of infection was with *Staphylococcus aureus* (12; 28.57%), followed by *Pseudomonas aeruginosa* (9; 21.43%), *Escherichia coli* (8; 19.05%), *Klebsiella pneumonia* (6; 14.29%) and *Proteus mirabilis* (4; 9.52%), while the lowest percentage of bacteria belonged to *Staphylococcus epidermidis* (3; 7.14%).

Table 1. Numbers and percentages of bacteria isolated from different clinical infections.

Source of sample / Bacteria	Urine	Sputum	Wounds	Total
S. aureus	٤ (25.00%)	3 (27.27%)	5 (33.33%)	12 (28.57%)
S. epidermidis	1(6.25%)	0 (0.00%)	2 (13.33%)	3 (7.14%)
E. coli	4(25%)	2 (18.18%)	2 (13.33%)	8 (19.05%)
P. aeruginosa	3(18.75%)	2 (18.18%)	4 (26.77%)	9 (21.43%)
K. pneumonia	1(6.25%)	3 (27.27%)	2 (13.33)	6 (14.29%)
P. mirabilis	3(18.75%)	1 (9.09%)	0 (0.00%)	4 (9.52%)
Total	16	11	15	42

The isolates of *Staphylococcus aureus* were considered as the highest infection rate among pathogenic gram-negative bacteria, followed by *Pseudomonas aeruginosa*. The isolates of *S. aureus* exhibited a difference in the rates of its isolation from different disease samples due to the variation in the disease samples under study.

Detection of the active groups' components of the biosurfactant

The qualitative tests showed that the biosurfactant contains many active groups (thanins, carbohydrates, Phenols, Flavonoids, Saponins) and does not contain triterpenoids (Table 2).

Table 2. Chemical detection of active groups of biosurfactant produced from S. *thermophilus* Isolated from iraqi local cheese.

No.	Chemical Detections	Result
1	Tannins	+
2	Carbohydrates	+
3	Phenols	+
4	Flavonoids	+
5	Fuocuomarins	+
6	Saponins	+
7	Triterpenoids	-

Antibacterial activity of biosurfactant, produced from *S. thermophilus*

The antibacterial efficacy of biosurfactant against *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *P. mirabilis* was estimated to confirm that it exhibits an antibacterial activity. Three different concentrations of biosurfactant including 25 - 50 - 100 mg mL^{-1} were taken and examined against bacterial isolates from different clinical infections to find out the inhibition efficiency for each concentration and according to the types of bacteria. The results of the statistical analyses are shown in Table 3. The significant increase was considered at the level of $p < 0.05$. The concentration of 100 mg mL^{-1} displayed the highest average diameter of inhibition zon (25 mm) for *S. epidermidis*, while the lowest (14.5 mm) belonged to *P. aeruginosa*. The concentration of 50 mg mL^{-1} exhibited the highest rate of inhibition zone (19 mm) for *S. epidermidis*, while the lowest (14 mm) belonged to *K. pneumoniae*. The concentration of 25 mg mL^{-1} revealed the highest average diameter of inhibition zone (15 mm) for *S. epidermidis*, while the lowest (10 mm) belonged to *P. aeruginosa* compared to the control factor containing sterile MRS broth (Table 3).

Table 3. Antibacterial activity of the biosurfactant against pathogenic bacteria isolated from different clinical infections.

Bacteria	Con.	Mean	SD	p-value
S. aureus	100 mg mL^{-1}	24.0^{a}	2.0	0.001**
	50 mg mL^{-1}	18.0^{b}	2.0	
	25 mg mL^{-1}	14.0^{c}	1.0	
S. epidermidis	100 mg mL^{-1}	25.0^{a}	1.0	0.000**
	50 mg mL^{-1}	19.0^{b}	1.0	
	25 mg mL^{-1}	15.0^{c}	2.0	
K. pneumoniae	100 mg mL^{-1}	16.0^{a}	1.0	0.037*
	50 mg mL^{-1}	14.0^{ab}	1.0	
	25 mg mL^{-1}	12.0^{b}	2.0	
E. coli	100 mg mL^{-1}	18.0^{a}	2.0	0.024*
	50 mg mL^{-1}	15.5^{ab}	2.0	
	25 mg mL^{-1}	12.0^{b}	1.7	
P. mirabilis	100 mg mL^{-1}	20.0^{a}	2.0	0.007*
	50 mg mL^{-1}	15.0^{a}	2.0	
	25 mg mL^{-1}	13.0^{b}	1.0	
P. aeruginosa	100 mg mL^{-1}	14.5^{a}	1.0	0.004*
	50 mg mL^{-1}	12.0^{b}	1.0	
	25 mg mL^{-1}	10.0^{c}	1.0	

Note: * = significant at $p < 0.05$, **significant at $p < 0.001$; The letters (a b c) represent the presence of significant differences between all three concentrations at the level of probability ($P < 0.05$) ($P < 0.001$). The letters (a ab c) represent significant differences between the first and third foci, as well as between the second and third, while the results did not show significant differences between the first and second foci at the level of probability ($P < 0.05$; $P < 0.001$).

The abovementioned results showed increases in the inhibitory activity of the biosurfactant at its concentration, as the inhibitory activity of lactic acid bacteria filtrates upraised significantly by elevation in its concentration (Sreekumar & Hosono 2000). The study also exhibited that the inhibitory activity of the biosurfactant against

gram-positive and gram-negative bacteria and yeasts increased by elevating its concentration. The results of the current study agree with the researchers' opinions about the inhibitory activity of *S. thermophilus* bacteria. As reported by Akpinar *et al.* (2011), *S. thermophilus* bacteria display inhibitory activity against *E. coli*, *P. fluorescens*, *K. pneumonia* and *S. aureus*. As it was explained by (Nurhajati *et al.* 2008), We also indicated that the biosurfactant produced from *S. thermophilus* bacteria isolated from Iraqi local white cheese has an inhibitory activity against all pathogenic bacteria under the current study due to its content of the inhibitor substances.

Effect of a biosurfactant of *S. thermophilus* on some biochemical parameters in albino male rats

The results of the statistical analyses are shown in Table 4 which depicts a significant decrease in total cholesterol and high-density lipoproteins LDL in the treatment group compared to control ($p<0.001$). A significant increase was also found in HDL in the treated group compared to control ($p < 0.05$).

Table 4. Effects of biosurfactant produced by *S. thermophiles* bacteria on some biochemical parameters.

Test	group	Mean	Std. Deviation	P-Value
CT (mg 100 mL^{-1})	C	78.51	1.07	0.000**
	TI	67.51	1.32	
TG (mg 100 mL^{-1})	C	85.33	0.45	0.000**
	TI	69.14	1.50	
HDL-C (mg 100 mL^{-1})	C	32.93	1.60	0.000**
	TI	43.08	1.83	
LDL-C (mg 100 mL^{-1})	C	53.76	1.12	0.000**
	TI	41.46	1.18	

**significant at $p < 0.001$.

According to the results obtained, it was indicated that the biosurfactant reduces the level of cholesterol in the blood serum. The decrease in serum cholesterol is due to the fact that the biosurfactant contains phenolic substances, which are antioxidants and work to reduce fat cells when they affect the cholesterol synthesis process, hence reduces its manufacture. Also, the antioxidant substance raises the good HDL-C and reduces the bad LDL-C.

CONCLUSION

In the present sudy, we found the ability to produce biosurfactant from *Streptococcus thermophilus* isolated in Iraqi local cheese and its antibacterial activity to eliminate some gram-positive and gram-negative bacteria isolated from different clinical samples. The biosurfactant has a significant effect on some biochemical parameters of male rats under the current study.

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