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Production of biosurfactant by lactic acid bacteria using whey as growth medium

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Abstract: The aim of this study was to produce biosurfactants from whey waste using *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Lactobacillus rhamnosus* as well as to determine oil spreading, emulsification index, surface tension, and antiadhesive properties in these biosurfactants. Additionally, the capability of biosurfactant production from whey waste in the dairy industry was compared with that of MRS broth, a commercial culture medium. The presence of biosurfactants by all lactic acid bacteria was detected using the oil spreading test. Zone diameter due to the surface activity of lactic acid bacteria strains ranged from 1.87 to 5.92 cm. Biosurfactants from both whey medium and MRS broth reduced surface tension. Differences between data from whey medium and MRS broth were statistically insignificant in terms of the biomass, oil spreading, and surface tension of biosurfactants. Emulsification index values recorded after 1 h, 24 h, and 1 week were significantly different and ranged from 19.50% to 58.00%. The highest emulsification activity was exhibited by *L. acidophilus* from whey medium in the first hours. A 10 mg/mL concentration of biosurfactants was able to prevent *S. aureus*, *P. aeruginosa*, and *E. coli* adhesion 37.25%–52.5%, 10.25%–23.25%, and 5.32%–11.50%, respectively. *E. coli* was more resistant to the biosurfactants than the other pathogens were. On the other hand, biosurfactants from *L. rhamnosus* had the lowest antiadhesive effects. In general, biosurfactants from whey medium and MRS broth were similar in terms of antiadhesion properties. The present study showed that dairy wastes could be an appropriate medium for cost-effective biosurfactant production by lactic acid bacteria for the benefit of the food, pharmaceutical, and cosmetic industries.

Key words: Whey, dairy industry waste, biosurfactant, lactic acid bacteria, antiadhesion

1. Introduction

Biosurfactants are amphiphilic compounds synthesized by microorganisms comprising distinct hydrophilic and hydrophobic moieties that can affect the surface or interfacial properties of a liquid [1]. There is increasing interest in biosurfactants because of their diversity, environmentally friendly nature, biological safety, the possibility of their production via fermentation, and their potential uses in various industrial fields such as the bioremediation, medical, pharmacological, and food processing industries [2,3]. Biosurfactants in the food industry have been applied as food emulsifiers, antioxidant agents, antibiofilm agents, antimicrobial agents, and antiadhesives [4]. Compared with chemical surfactants, biosurfactants have numerous advantages due to their lower toxicity and higher biodegradability, better environmental compatibility, high selectivity, and effectiveness at extreme temperatures, salinities, or pH [5]. However, the drawbacks of biosurfactants, compared with synthetic surfactants, are low productivity and high production cost. Thus, a method to ensure biosurfactant production at low cost and with higher yield is essential [1,6]. Cost-effective production of biosurfactants could be achieved using food industry waste or other industrial effluents [2,5].

The choice of cheap raw materials is the most important issue for the overall economics of biosurfactant processing. The dairy industry creates significant amounts of by-products such as butter milk, whey, and their derivatives. Whey, a fluid by-product of cheese processing, consists of lactose (75% of dry matter), protein (12%–14%), organic acids, minerals, and vitamins [7,8]. There are disposal challenges mainly from whey in the dairy industry. Degradation of whey with simple and economical solutions overcomes the main pollution problem from the dairy processing field. Additionally, there are recent studies based on the effective utilization of whey as cheap fermentation medium for the production of biosurfactant [9].

Pseudomonas spp. and *Bacillus* spp. are the species mainly used for biosurfactant production [6]. However, recent papers revealed lactic acid bacteria are good

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biosurfactant producers. Lactic acid bacteria reported as biosurfactant producers consist of *Lactobacillus* strains (*L. helveticius*, *L. pentosus*, *L. lactis*, *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. fermentum*, *L. casei*, *L. paracasei*, *L. jensenii*, *L. reuteri*, *L. gasseri*, *L. delbrueckii*, *L. gallinarum*, *L. amylovorus*, and *L. crispatus*), *Bifidobacterium* strains (*B. adolescentis*, *B. animalis*, *B. bifidum*, *B. infantis*, *B. longum*, *B. essencis*, *B. breve*, and *B. lactis*), and other lactic acid bacteria (*Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus thermophilus*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, and *Lactococcus lactis*) [1,10–14].

Biosurfactants synthesized by lactic acid bacteria could attract the attention of the food industry because of their compatible emulsifying and antiadhesive activities. In particular, biosurfactants produced by lactic acid bacteria are recognized for their beneficial properties. However, further studies are needed on the economical production of biosurfactants [14]. The present study aimed to produce biosurfactants by lactic acid bacteria using whey as well as to investigate some properties of these biosurfactants.

2. Materials and methods

2.1. Microorganisms, medium, and food waste

Lactobacillus rhamnosus (Ezal, commercial starter culture), *Lactobacillus acidophilus* NCC 36, and *Streptococcus thermophilus* NCC 2290 were used for biosurfactant production. *Escherichia coli* K12, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 27853 (Department of Biology, Çukurova University) were used as indicator (target) microorganisms to detect the antiadhesive characteristics of the biosurfactants. While MRS agar and broth (Merck, Germany) were used for the growth and stock solutions of *L. rhamnosus* and *L. acidophilus*, M17 agar and MRS broth were used for the growth and stock solutions of *S. thermophilus*. Indicator microorganisms were growth in Trypticase soy broth (TSB) (Sigma-Aldrich) and stored at –20 °C in TSB supplemented with 20% (v/v) glycerol (Merck) [15].

To produce biosurfactant by lactic acid bacteria, whey was supplied as a substrate from the dairy processing plant in Çukurova University, Agriculture Faculty, Research and Application Farm.

2.2. Preliminary analysis and pretreatments in whey

Before the biosurfactant production and related analysis, lactose and protein amounts in the whey were determined and then the whey to be used in the production of biosurfactant was subjected to some pretreatments. The amounts of sugar and protein in whey were respectively determined by the Lane–Eynon method [16] and Kjeldahl method [17]. As a pretreatment for the production of biosurfactant in whey, whey was adjusted to pH 4.5 using 5 N HCl and then autoclaved for 15 min at 121 °C to denature the proteins. Sterilized whey was centrifuged at 4 °C and 6500 × *g* for 10 min. Thus, the supernatant obtained by removing precipitates was sterilized for 15 min at 121 °C and adjusted to pH 6.7 for use as whey medium.

2.3. The production of cell-free supernatant and the extraction of biosurfactant from whey

Overnight cultures of lactic acid bacteria (15 mL) were inoculated into 600 mL of whey medium and MRS broth (control) in flasks. These mixtures were incubated for 48 h at 37 °C with 150 rpm agitation and then centrifuged (6500 × *g* at 4 °C, 20 min) for separation of the cell-free culture supernatant (CFS). This CFS was stored at +4 °C for use in the biosurfactant analysis [18–21].

For biosurfactant extraction, ethyl acetate precipitation was used. CFS was adjusted to pH 2 using 6 M HCl and then transferred into a separatory funnel and an equal amount of ethyl acetate was added. The mixture was shaken for phase separation with three replications. After phase separation, the collected organic phase was treated with anhydrous sodium sulfate to remove water and then concentrated on a rotary evaporator at 40 °C to obtain crude biosurfactant extract. The level of biosurfactant production by lactic acid bacteria was evaluated by measuring this biosurfactant extract in g/L [22].

2.4. The detection of biosurfactant presence by oil spreading technique

First 10 µL of crude oil (one drop) (Mersin ATAŞ Petroleum Refinery) was dropped into the center of a petri dish (diameter 9 cm) containing 25 mL of distilled water. Then 20 µL of CFS was added to the middle of the crude oil and the diameter of the zone from supernatant was measured in centimeters. These zone diameters were compared with zone diameters from Tween-80 as a positive control sample. The diameter of the transparent zone (cm) was evaluated as "+" for 0.5–0.9 cm, "++" for 1–1.5 cm, "+++" for 1.5–2.1 cm, and "++++" for 2.1 cm [23–25].

2.5. The measurement of emulsification index and surface tension

The biosurfactant emulsification index assay was performed with a slight modification according to Ramnani et al. [26]. Water, xylene, and CFS were mixed in 20:30:10 ratio in a graduated cylinder and the height of the solvent layer in the graduated cylinder was recorded. Then an emulsion was obtained by vortexing the mixture. The height of the emulsion layer was recorded after 1 h, 24 h, and 1 week. The following equation was used for calculation of the emulsification index: EI (%) = [(height of emulsion layer)/(height of oil + emulsion layer)] × 100.

The surface tension of the cell-free culture supernatants was measured according to the ring method by a tensiometer, TD1C-LAUDA [27].

2.6. Antiadhesive activity of biosurfactant

For the determination of antiadhesive activity, a 96-well microtiter plate was filled with 200 µL (2.5 mg/mL,

5 mg/mL, 10 mg/mL) of the biosurfactant extract and 200 µL of the control samples with distilled water and it was incubated at room temperature for 24 h to achieve attachment of biosurfactant extract. Then the cells were carefully washed 3 times with 200 µL of distilled water. The microtiter plate previously coated with biosurfactant was filled with 180 µL of TSA and incubated at 35 °C for 10 days by adding of 200 µL of pathogenic bacteria (10^9 cfu/mL). At the end of the incubation, the microtiter plate wells were washed with distilled water and then were kept with a solution of 200 µL of methanol for 15 min, followed by 15 min with crystal violet (1%, g/L). Then it was filled with 200 µL of 33% (v/v) acetic acid. Finally, the optical density was measured at 600 nm using an ELISA microtiter plate reader and percent of microbial inhibition (%) was calculated from these absorbance values. Microbial inhibition (%) = [1 – (Ac/Ao)] × 100; Ac: the absorbance value of biosurfactant cells; Ao: the absorbance value of control cells [15,28].

2.7. Statistical analysis

Statistical analyses were performed using Windows SPSS 15.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to compare differences in significance among the trials and Duncan's multiple comparison test was used to compare differences between the groups ($P < 0.05$) [29].

3. Results

In the present study, some properties related to biosurfactants from whey medium were compared with those of MRS broth as a control sample. Additionally, differences or similarities among lactic acid bacteria species in terms of capability of biosurfactant production were revealed.

3.1. The physicochemical properties of biosurfactants

As seen in Table 1, all lactic acid bacteria strains showed high surface activity with zone diameters ranging from 1.87 to 5.92 cm. As a result of the high zone diameters, the presence of biosurfactant was detected with mostly "++++" points. This situation indicated that oil spreading values from the biosurfactants in the present study were similar to oil spreading values (7.5 cm, ++++) from Tween-80 chemical surfactant.

Biomass values of the biosurfactants are shown in Figure. Biomass values (between 9.20 and 11.80 g/L) from whey medium were higher than those from MRS broth (between 6.38 and 8.20 g/L). The lowest biomass values in terms of lactic acid bacteria species were obtained from *L. rhamnosus*. On the other hand, all data relating to biomass were statistically insignificant. In the present study, before lactic acid bacteria inoculation, surface tensions were measured as 50 mN/m for MRS broth and 72 mN/m (based on pure water) for whey medium. After lactic acid bacteria inoculation into whey medium and MRS broth, surface tension is expected to decrease. This hypothesis was confirmed by the present results as surface tension values of biosurfactants from whey medium ranged from 48.85 to 53.51 mN/m, whereas biosurfactants from MRS broth resulted in lower surface tension values between 43.44 and 45.35 mN/m. However, similar to the biomass results, all data from the surface tension test were statistically insignificant with regard to cultivation medium and lactic acid bacteria species. Additionally, a positive correlation was observed between biomass values and surface tension values (see Figure and Table 2).

Emulsification index values from lactic acid bacteria species were compared in different cultivation conditions. As observed in Table 3, emulsification stability was retained throughout 168 h. In general, *L. acidophilus* showed the highest resistance due to its time-dependent stability. Additionally, the highest emulsification index value was obtained with biosurfactants of *L. acidophilus* in whey medium. Differences between the emulsification index values from MRS and from whey medium were statistically insignificant. In terms of lactic acid bacteria species, there were no differences between the emulsification index values of *L. acidophilus* and *S. thermophilus*. On the other hand, the emulsification index values of *L. rhamnosus* were significantly lower than the others.

3.2. Antiadhesive properties of biosurfactants

In the present study, the antiadhesion effects of biosurfactants at levels of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL were tested. Table 4 represents the antiadhesion capability of biosurfactants against pathogens. Biosurfactant at a concentration of 2.5 g/L had no antiadhesion ability against pathogens, except *S. aureus*. However, 10 mg/mL biosurfactants as the

Table 1. Oil spreading values of biosurfactants (cm).

	L. acidophilus	*L. rhamnosus*	*S. thermophilus*
Biosurfactants from whey medium	3.60 ± 0.33	1.87 ± 0.47	2.87 ± 0.47
Biosurfactants from MRS broth	4.87 ± 0.35	5.92 ± 0.29	5.00 ± 0.40

There are no differences between averages ($P < 0.05$)

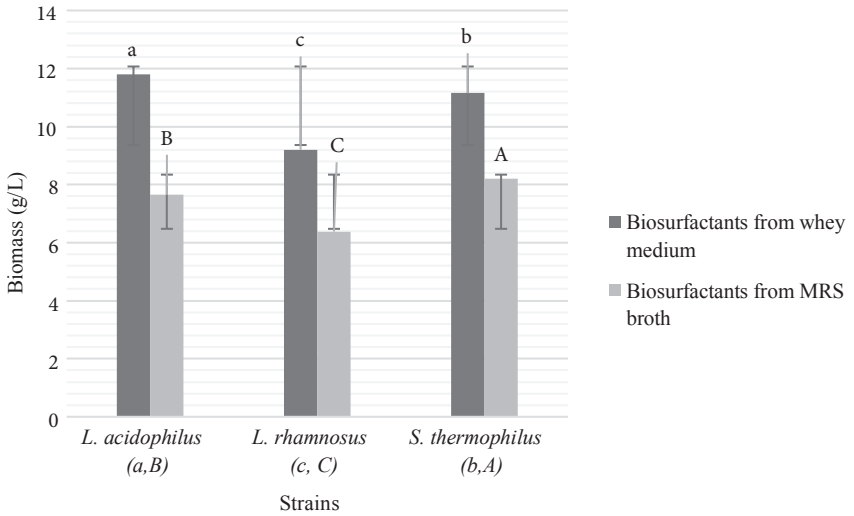

Figure. Biomass values of biosurfactants (a, b, c: different alphabetical letters indicate whey groups that are significantly different; A, B, C: different alphabetical letters indicate MRS broth groups that are significantly different).

Table 2. Surface tension values of biosurfactants (mN/m).

	L. acidophilus	*L. rhamnosus*	*S. thermophilus*
Biosurfactants from whey medium	53.51 ± 5.29	52.45 ± 8.27	48.85 ± 4.16
Biosurfactants from MRS broth	43.44 ± 1.06	45.35 ± 2.39	43.69 ± 3.22

There are no differences between averages ($P < 0.05$)

Table 3. Emulsification index values of cell-free supernatant from lactic acid bacteria (%).

Hours	*L. acidophilus*		*L. rhamnosus*		*S. thermophilus*	
	Whey	MRS broth	Whey	MRS broth	Whey	MRS broth
1	$58.00^{bC} \pm 1.63$	$41.75^{bC} \pm 1.50$	$39.25^{aC} \pm 1.70$	$40.75^{aC} \pm 1.71$	$54.75^{bC} \pm 1.26$	$44.75^{bC} \pm 1.26$
24	$50.50^{bB} \pm 1.00$	$33.25^{bB} \pm 2.36$	$33.00^{aB} \pm 1.50$	$33.25^{aB} \pm 2.22$	$48.25^{bB} \pm 1.50$	$37.50^{bB} \pm 0.57$
168	$32.50^{bA} \pm 1.73$	$20.25^{bA} \pm 1.70$	$20.75^{aA} \pm 1.50$	$19.50^{aA} \pm 1.00$	$26.75^{bA} \pm 2.75$	$24.75^{bA} \pm 0.95$

a, b, c: The averages shown on the same line with different exponents are significantly different.
A, B, C: The averages shown in the same column with different exponents are significantly different ($P < 0.05$)

most antiadhesive dose was able to prevent *S. aureus*, *P. aeruginosa*, and *E. coli* adhesion 37.25%–52.5%, 10.25%–23.25%, and 5.32%–11.50%, respectively. This situation indicated that there was a necessity for larger amounts of biosurfactant for complete inhibition (100%). The highest antiadhesion effect was observed against *S. aureus* and this was followed by *P. aeruginosa* and then *E. coli*. Differences between the antiadhesive effects of biosurfactants from whey medium and MRS broth were statistically insignificant. Additionally, biosurfactants produced by *S. thermophilus* resulted in the most antiadhesion effect for all pathogens tested in the present study, similar to the emulsification index results. This situation showed that there is a positive correlation between emulsification index values and antiadhesive properties. The least antiadhesive biosurfactants were produced by *L. acidophilus* against *S. aureus* and *P. aeruginosa* and by *L. rhamnosus* against *E. coli*. These results showed that the antiadhesion effect was dependent on the lactic acid bacteria species from which the biosurfactants were obtained.

Table 4. Antiadhesion effects of biosurfactants at different concentrations against pathogenic bacteria (% inhibition).

Pathogens	Biosurfactant (mg/mL)	*L. acidophilus*		*L. rhamnosus*		*S. thermophilus*	
		Whey	MRS broth	Whey	MRS broth	Whey	MRS broth
E. coli	2.5	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}
	5	2.75^{b}	3.17^{b}	1.82^{a}	0.62^{a}	3.42^{c}	3.75^{c}
	10	8.25^{b}	11.25^{b}	5.32^{a}	7.25^{a}	11.50^{c}	10.25^{c}
S. aureus	2.5	7.12^{b}	4.50^{b}	5.32^{a}	2.50^{a}	8.62^{c}	5.75^{c}
	5	39.75^{b}	31.50^{b}	36.25^{a}	24.75^{a}	42.00^{c}	34.75^{c}
	10	45.25^{b}	41.75^{b}	45.37^{a}	37.25^{a}	52.50^{c}	47.25^{c}
P. aeruginosa	2.5	0.00^{b}	0.00^{b}	0.00^{a}	0.00^{a}	0.00^{c}	0.00^{c}
	5	10.25^{b}	6.00^{b}	6.75^{a}	2.12^{a}	11.25^{c}	10.50^{c}
	10	19.75^{b}	15.50^{b}	20.50^{a}	10.25^{a}	23.25^{c}	16.75^{c}

a, b, c: The averages shown on the same line with different exponents are significantly different ($P < 0.05$)

4. Discussion

Whey, with its high compositional properties including dry matter, lactose, and protein amounts, has potential for use as a substrate to produce biosurfactants [7,8]. In the present study, the amounts of lactose and protein in whey were 4.5% and 0.9%, respectively. In previous studies based on the use of whey in biosurfactant production, lactose and protein amounts were respectively 6.5%–7.5% and 11%–14% [18,30]. The present results with regard to lactose and protein content are lower than the results in the literature.

Researchers mainly concentrated on *Pseudomonas* and *Bacillus* species for biosurfactant studies but recent papers revealed biosurfactants produced using lactic acid bacteria [1,4,7]. The potential of lactic acid bacteria regarding biosurfactant production is dependent on cultivation conditions (growth medium composition, temperature, growth phase of culture, aeration, agitation, and pH) and species [6]. As is known, MRS broth is a selective and optimal growth medium for lactic acid bacteria [11,12]. On the other hand, previous papers highlighted that whey is an excellent growth medium for various types of microorganisms as an alternative to commercial culture media [9]. In the present study, biosurfactants extracted by lactic acid bacteria from whey medium were compared with biosurfactants from MRS broth. Although the biosurfactants in the present study were extracted at different levels between 6.38 and 11.80 g/L by lactic acid bacteria from these growth media, these differences were statistically insignificant. In the study by Rodrigues et al. [31], biosurfactant production of *S. thermophilus* and *Lactococcus lactis* was optimized by adding various supplements to the cultivation medium. Growth medium-associated biosurfactant production was also supported in the present study. In the present study whey medium resulted in more efficiency in terms of activity of biosurfactants synthesized by *L. rhamnosus*. Golek et al. [11] performed biosurfactant production in high yield and with antiadhesive properties from whey medium using *L. casei*. In another study, while *Lactobacillus agilis* produced biosurfactants of 8.4 g/L in MRS broth, biosurfactant production of *L. agilis* by using whey medium resulted in higher yield of 9.60 g/L [32]. Potowary et al. [33] reported that a yield of 2.7 g/L crude biosurfactant was obtained by solvent extraction of the supernatant medium of *P. aeruginosa* grown in whey medium with ethyl acetate. Interestingly, *P. aeruginosa*, the most popular biosurfactant producer, gave lower yield than lactic acid bacteria in the present study. This situation indicated that lactic acid bacteria used whey more effectively than *Pseudomonas* species did.

As is known from the literature, the oil spreading test detects the ability of microbial strains related to the production of biosurfactants [12]. Oil spreading values in the present study were quite high (mostly ++++). In the study by Kaur et al. [5] based on biosurfactants of lactic acid bacteria, the values of oil spreading were between 0.2 and 1.5 cm. As observed, our results are more favorable. The oil spreading analysis is performed as an indirect measurement of surface activity of biosurfactants in which a larger zone diameter is correlated with higher surface activity [14]. Lactic acid bacteria have the capability of oil spreading, which demonstrates the presence of biosurfactant [5,14]. Kaur et al. [5] reported that biosurfactants from lactic acid bacteria such as *L. acidophilus*, *L. plantarum*, and *Lactobacillus fermentum* resulted in displacement from 0.2 cm to 1.5 cm with their high surface activity. In another report, biosurfactants produced by *L. plantarum* resulted

in high oil spreading values (approximately 35 mm) after 48 h cultivation in MRS broth [14]. In the present study, the oil spreading activities of all strains were positive and the maximum oil spreading activity was exhibited by *L. acidophilus* and *S. thermophilus*. In accordance with the present study's results, previous reports confirmed the production of biosurfactants by *L. acidophilus*, *L. rhamnosus*, and *S. thermophilus* [9].

Emulsification supports the consistency, texture, phase dispersion, and the solubilization of flavor in food industry products. Biosurfactants may ensure adequate emulsification by stabilizing the microscopic droplets. Cultivation conditions such as growth medium compositions, incubation period, microbial strains, and medium temperature could influence emulsification activity. In particular, emulsification index decreased over time. Our results were also in accordance with this [4]. Emulsification activity could also change according to the hydrocarbon used in related analyses [34]. In our study, emulsification index values were obtained from xylene, but in future studies different hydrocarbon substrates should be tested for establishment of better correlations. For example, Satpute et al. [34] reported that the emulsification index value of biosurfactant from *Lactobacillus* spp. using xylene was approximately two times lower than that using heptane. Emulsifying and dispersing agents used in food products do not have to decrease the surface tension of water or of hydrocarbons. That means that ingredients utilized for the aim of emulsification and dispersing do not have any obligation to reduce surface tension. Thus, in some circumstances, while biosurfactants to reduce surface tension could exhibit high power, emulsification properties of these biosurfactants could be poor [4]. In accordance with this literature information, in the present study the biosurfactant of *L. acidophilus* resulted in the highest emulsification index but could not achieve the highest reduction in surface tension.

According to the ring method, surface tension was determined by evaluating the surface tension of pure water (72 mN/m). An effective biosurfactant should decrease this value to 30 mN/m [1,5]. Various factors such as pH, salinity, and temperature that are effective on biosurfactant activity also affect the surface tension properties of the biosurfactant. As the efficiency or concentration of biosurfactant increases, surface tension decreases [12,35]. Previous studies highlighted that biosurfactants from lactic acid bacteria are highly effective in reducing surface tension [32]. The surface tension values of biosurfactants from lactic acid bacteria generally varied from 41.8 mN/m to 57.6 mN/m [13,20,32,36]. For example, Vera et al. [36] compared biosurfactants produced by *Lactococcus lactis* in different cultivation media including MRS broth and whey in terms of reduction of surface tension. In their studies, while surface tension values from whey and MRS broth were 49.3 mN/m and 49.1 mN/m, respectively, there were no significant differences between MRS broth and whey. Similarly, differences among surface tension values in the present study were statistically insignificant in terms of both species and cultivation medium.

Adsorption of biosurfactants to solid surfaces provides several advantages for not only in the medical field, but also in food plants by preventing microbial adhesion and fighting bacterial colonization [37]. Bacterial biofilms are more resistant to disinfectants than their planktonic form. Therefore, when novel antimicrobials were evaluated for use of industry, their effects on biofilms or antiadhesive potential were taken into consideration [10,12,20]. Most literature on biosurfactants revealed antiadhesive activities of biosurfactants against *Listeria monocytogenes*, *S. aureus*, *E. coli*, *Micrococcus luteus*, *Pseudomonas* spp., and *Bacillus* spp. [4]. The biosurfactants from the present study exhibited antiadhesive activity against all tested pathogenic bacteria such as *E. coli*, *S. aureus*, and *P. aeruginosa*. However, the values for antiadhesive effect were different depending on the pathogen bacteria species tested. The antiadhesive activity was proportional to the amounts of biosurfactant [18,31]. In accordance with this information, the present paper detected that 10 mg/mL biosurfactant led to more antiadhesion than 5 mg/mL. For instance, Gudina et al. [20] reported that the minimum bactericidal concentration of biosurfactants from *Lactobacillus paracasei* to control *E. coli* and *S. aureus* was between 25 and 50 mg/mL. Additionally, the most effective antiadhesive properties (70%) were exhibited against *S. aureus* in their study.

The antiadhesive effects of biosurfactants synthesized by *Lactobacillus* species including *L. acidophilus*, *L. rhamnosus*, *L. casei*, and *S. thermophilus* are well documented against pathogenic microorganisms including *E. coli*, *S. aureus*, *Staphylococcus epidermidis*, *Salmonella* spp., *Streptococcus agalactiae* etc. [20,35]. Inhibitory activities of biosurfactants could differ according to the substrates or sources and microbial species used in their production and also species of the target pathogenic bacteria [6,8]. The present study supported this hypothesis. In general, previous reports detected that biosurfactants of *L. rhamnosus* exhibited lower antiadhesive properties than *L. acidophilus* in accordance with our results. According to the literature, the lowest inhibitory activity was exhibited against *E. coli*, similar to the present study's results [7,10,20,34,38]. For instance, Gudina et al. [32] stated that the biosurfactant from *L. agilis* showed significant antiadhesive effect against *S. aureus*, *S. agalactiae*, and *P. aeruginosa*. Rienzo et al. [38] reported that biosurfactants exhibited more antibacterial activity against *S. aureus* than *P. aeruginosa* and *E. coli*.

In conclusion, the present study highlighted that *L. rhamnosus*, *L. acidophilus*, and *S. thermophilus* as biosurfactants producer strains achieved the utilization of whey from dairy wastes and exhibited emulsifying, inhibitory, and antiadhesive properties. In future studies, the optimization of the biosurfactant production process by different microbial species from whey medium should be attempted to achieve high yield and low extraction costs. As whey waste could economically be converted to biosurfactant, successfully commercialized and more diversified novel biosurfactants could be detected in the market.

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