Indian Journal of Natural Products and Resources
Vol. 14(2), June 2023, pp. 324-331
DOI: 10.56042/ijnpr.v14i2.4218

Short Communication

Enhancing the shelf life of raw unprocessed milk by bacterial biosurfactants extracted from indigenous microbes present in cow's milk

E. Mohanachitra, R. Menaka and S. Karthik Sundaram*

Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore 641048, Tamil Nadu, India

Received 15 August 2020; revised received 06 December 2022; accepted 17 January 2023

Biosurfactants are microbial amphiphilic compounds, which can reduce surface tension between two immiscible liquids. Indigenous microbes isolated from raw unprocessed cow's milk were characterized morphologically and biochemically. The biosurfactant-producing ability of the isolates was determined by hemolytic activity, oil displacement technique and their emulsification activity which screened against oil. Biosurfactants were produced and those were tested for their antibacterial activities against various bacterial strains. Then the biosurfactants were added to milk samples for analysing the shelf life of raw unprocessed cow's milk. The effect of biosurfactants on raw milk is analyzed by visual and microbial enumeration. In this study, 8 indigenous microbes were isolated from raw milk. The best isolates for biosurfactant production were identified as *Bacillus* sp., and *Lactobacillus* sp., based on screening assays. The biosurfactant extracted from these screened isolates exhibited antimicrobial activity against infectious agents. Moreover, they showed its effect on the potential enhancement of the shelf life of raw milk. Isolated and screened microflora can be a good source of biosurfactant and they are proven to be a beneficial means for large and small-scale industries to supply good quality milk.

Keywords: Antagonistic activity, Biosurfactant, Cow's milk, Microbial surfactant, Shelf life of milk, Sustainability

IPC code; Int. cl. (2021.01)- A23C

Introduction

Milk is a highly nutritious food for human beings across the globe. A number of animals are used to produce milk for human consumption, although the cow is by far the most important in commercial terms. However, due to various sources of direct and indirect contamination, various chemical and microbiological contaminants are found in cow's milk[1]. Milk contains four principal components of water, fat, protein and lactose, which constitutes more water than any other element, around 87% for dairy cows. Milk serves as a good source of calcium, vitamin D, proteins and essential nutrients and also provides phosphorous, potassium, magnesium and various vitamins viz. vitamin A(retinol), vitamin B12 (cyanocobalamin), and riboflavin. In addition, a variety of diseases are potentially transmissible through milk they are caused by the presence of microbes and those microbes play both harmful and beneficial to human beings. There are three types of microorganisms occurring in milk, which are biochemical, temperature characteristic and pathogenic types. The biochemical group consisting of microbes are acid-forming, Gas forming, Ropy-milk forming, proteolytic and lipolytic microbes that bring biochemical changes in milk[2].

Milk has only beneficial microbes while collecting milk from the udder. The source of disease-causing microorganisms occurring in milk via dairy cattle, air, feedstuffs, milk handling equipment and the milker[3]. Another way of entering microorganisms is that the vendors add water to increase the quantity of the milk. By chance, if the water has some bacteria, algae, protozoa and cyanobacteria. Bacterial types commonly associated with milk are *Bacillus* (hydrolyse milk protein), *Streptococcus thermophilus* (acid fermentation), *S. agalactiae* (pathogenic), *S. lactis* (acid fermentation), *Lactobacillus lactis* (acid production), *L. bulgaricus* (acid production), *L. Acidophilus* (acid production), Propionibacterium (acid production) and *S. lactis-diacetyllatic* (flavour production). India stands in the first of producing vast quantity of milk (150 million tonnes). Even though production of milk increased but it is not enough for all the people because of unstability. This unavailability has been prevailing, to short out the issue help of microbes needed. It will soon curl out through the action microbes that are normal and contaminants while collecting milk from the cow[4].

Bacteria and microscopic fungi produce extra cellular products like biosurfactants with many properties and applications[5]. A few biosurfactant-producing bacteria are named by Aminu *et al.*[6] and they are, *Acinetobacter, Arthrobacter, Agrobacterium, Antarctobacter*, *Bacillus, Clostridium, Lactobacillus, Halomonas, Serratia*, and *Rhodococcus*. Sanjana *et al.*[7], mentioned the names of species that produce biosurfactants most in the *Bacillus* genus.

*Correspondent author
Email: karthiksundaram@drngpasc.ac.in

They are *B. subtilis, B. polymyxa, B. Licheniformis* and *B. pumilus.* Surfactants are amphiphilic surface-active agents possessing hydrophobic and hydrophilic portions[8]. They act between fluids of different polarities such as oil/water and water/oil, which causes a reduction in surface tension, an increase in the area of contact of insoluble compounds (hydrocarbon) and the enhancement of the mobility, bioavailability, and biodegradation of such compounds[9]. There are different groups of biosurfactants; they are glycolipids, particulate biosurfactants, polymeric biosurfactants, fatty acids, neutral lipids and phospholipids[10].

Biosurfactants are produced in aqueous media with a carbon source feedstock such as carbohydrates, hydrocarbons, fats and oils by mainly aerobic microorganisms[11]. Biosurfactants are produced by microbes which are secreted into the culture medium by facilitating the translocation of insoluble substrates across cell membranes[12,13]. Based on molecular weight and microbial origin, composition, biosurfactants are classified[14]. They have anti-microbial, anti-adhesive, tolerance to high pH, temperature, biodegradability etc. Hence, this research is designed to isolate biosurfactant-producing bacteria from raw unprocessed cow's milk and determine their antimicrobial susceptibility pattern thereafter which isapplied in food samples to control the spoilage by inhibiting the growth of microorganisms present in the food.

Materials and Methods

Sample collection

The milk (cow milk) sample was collected in March 2021 from a milk centre located at Rakkiyapalayam, Avinashi, Tirupur (11°09'22.9"N 77°16'43.7"E). One litre quantity of six milk samples was collected in separate sterile bottles. The samples were collected in raw unprocessed conditions, then only the microbes were isolated. After collection, all samples were labelled appropriately and kept in the insulated box then transported to the laboratory. All samples were processed before the milk samples were spoiled.

Isolation of bacteria

Direct isolation of the microorganisms was carried out using the serial dilution technique of different milk samples in normal saline. Serial dilutions were plated onto the nutrient agar using the spread plate method. The cultures were incubated at 37°C for 24 h. Morphologically distinct colonies of pure form were isolated and identified[11].

Characterization of isolates

All of the pure cultures isolated from dairy samples were examined for their morphological, cultural and biochemical characteristics[15].

Morphological examination

The morphological examination was implemented by examining the colony morphology characteristics of the isolates.

Culture and biochemical characterization

Gram staining, motility test, endospore test, catalase, and sugar fermentation test were carried out to examine the cultural and biochemical characterization of all isolates.

Culture medium and bacterial growth

One mL of each isolated bacterial culture was inoculated separately in a 500 mL Erlenemeyer flask containing 150 mL of sterilized mineral salt medium with 1% crude oil (w/v). All flasks were kept in an incubator shaker for 7 days at 200 rpm, 30-37°C. After 7 days of the incubation period, the culture broth was centrifuged at 8000 rpm and 4°C for 10 min. The supernatant was collected through millipore filter paper which was used subsequently for screening methods such as drop collapse assay, oil spreading assay and emulsification assay. Moreover, the bacterial cells are also used for hemolytic assay[16].

Screening of biosurfactant-producing bacteria

Preliminary screening

Hemolytic activity, oil displacement test and drop collapse tests were used to screen the biosurfactant producers[9].

Hemolytic activity

The Hemolytic activity test was described by Rayeni and Nezhadin[17]. In brief, 50 µL of bacterial supernatant were inoculated in 5% sheep blood agar plates and incubated for 48 h at 37°C. After incubation, the plates were visually observed for the presence of a clear zone around the colony. The diameter of the clear zone was measured, which is used as an indicator of biosurfactant production.

Drop collapse method

Screening of biosurfactant production was performed using the drop collapse method. Ten microlitres of crude oil were added to 96 well microplate. To this, 10 µL of each culture supernatant was added and a drop shape on the oil surface was

observed after 1 min. The drop is flat. It was considered the result is positive for biosurfactant production. Instead, the drop is rounded which is considered as negative and it indicates the lack of biosurfactant production.

Oil spreading technique

One mL of distilled water was added to a clean grease-free microslide. To this 20 µL of crude oil was added and making a thin layer. A 10 µL of supernatant was then added to the oil surface. Instead of supernatant, 10 µL of distilled water was added to the oil surface which acts as a control. The diameter of the clear zone on the oil surface indicates surfactant activity that was calculated and it was compared with the control.

Complementary screening

Bacterial isolates gave positive for atleast one preliminary screening method that was subjected to the complementary assays to assure their ability to produce biosurfactant[9].

Emulsification activity

In this study, crude oil was used as the hydrophobic substrate. One mL of cell-freeculture broth was added to a 30 mL screw-capped test tube containing 5 mL of 50 mM Tris buffer (pH 8.0). After that, 5 mg of hydrocarbon was added to the solution and it was vortex for 1 min. This emulsion mixture was kept for 20 min. The emulsion mixture's optical density was measured at 610 nm on a spectrophotometer.

Biosurfactant recovery

In 500 mL of mineral salt medium, 1 mL of bacterial culture was added. It was incubated for 7 days at 35°C. After incubation, it was centrifuged at 8000 rpm for 15 min to recover the supernatant. The supernatant was collected and pH was adjusted to 2 using 6N HCl.Then this solution was stored at 4°C for 24 h for to precipitate the biosurfactant. To this, chloroform and methanol (2:1 v/v) mixture was added and mixed well. The organic layer contains precipitated biosurfactants which is recovered by separating funnel and dried[17].

Antibacterial activity of the extracted biosurfactant

Agar disk diffusion method was used to evaluate the *in vitro* antibacterial activities of extracted biosurfactants.Four dilutions (5, 10, 15, 20 mg/mL) of biosurfactants were prepared with double distilled water and indicator bacterial cultures spreaded on the mullerhinton agar plates. Then, discs containing the biosurfactants are placed on the agar surface which is incubated at 37°C for 24 h. Control experiments were carried out by using antibiotic under similar condition. After incubation, the diameters of inhibition growth zones around the discs were measured[9].

Testing the shelf life of raw unprocessed milk by biosurfactants

Concentration of biosurfactant was determined based on the Agar disk diffusion method. Extracted biosurfactants were added into the sterile flask containing milk sample on the ratio of 10%(v/v) to inspect the biosurfactant activity in enhancement of raw unprocessed milk's shelf life. Afterwards, microbial load enumerated for 4 days at the interval of 24 h in room temperature. For microbial enumeration, plate count agar and MRS agar was used for total aerobic flora and lactic acid bacteria respectively. The flask containing raw milk without biosurfactant in sample served as control. Moreover, the shelf life of milk samples was visually analyzed. After few days the results were observed and compared with normal milk sample. Sigmaplot14.0 (Trial version) software package have been used for draw the graphs.

Results and Discussion

Isolation of microbes

From the collected milk samples, 8 different bacterial colonies were isolated from the nutrient agar plates by diluting those collected milk samples. Table 1 shows the number of colonies and their growth on nutrient agar plates. There is out of 8 isolates, three isolates showed minimal growth followed by two with moderate growth and three with maximal growth which are mentioned as +, ++ and +++ respectively.

Characterization of isolates

Morphological examination

Table 2 shows the results of morphological characteristics such as type of colony, colour, margin,

Table 1 — Isolation of bacteria from milk samples

S.No	Isolate No.	Growth on nutrient agar
1	C1	+
2	C2	++
3	C3	++
4	C4	+++
5	C5	+
6	C6	+++
7	C7	+
8	C8	++

C = colony, + = Minimum growth, ++ = Moderate growth, +++ = High growth

Table 2 — Morphological characteristics of cultures isolates from milk samples

S.No	Colony No.	Type of colony	Colony colour	Margin	Pigment
1	C1	Small	Creamy white	Entire	_
2	C2	Small	Off white	Entire	_
3	C3	Large	Off white	Entire	_
4	C4	Large	Yellow	Entire	+
5	C5	Small	White shiny	Entire	_
6	C6	Large	Creamy white	Entire	_
7	C7	Small	White	Entire	_
8	C8	Large	Creamy white	Entire	_

+ = pigment formation, - = no pigment formation

Table 3 — Cultural and biochemical characteristics of pure isolates from milk samples

Colony No	Gram reaction	Morphology	Motility test	Endospore test	Catalase test	Sugar fermentation
1	+	Rods	+	+	+	Homo
2	+	Rods	+	_	+	Homo
3	+	Rods	_	_	_	Hetero
4	+	Rods	_	_	_	Homo
5	+	Rods	_	_	_	Homo
6	+	Cocci	_	_	_	Homo
7	+	Cocci	_	_	_	Hetero
8	_	Rods	+	_	+	Homo

+ = Activity present, - = Negative, homo = homofermentative, hetero = heterofermentative

and pigment production. All the isolated colonies appeared small and large in shape. The colour of colonies ranged from off-white, yellow, shiny white and creamy white. Entire margins were obtained for all the isolated colonies. Further, the colour pigments were present in only one type of colony (C4) and were absent in the remaining colonies.

Cultural and biochemical characterization

Various cultural and biochemical tests were studied for the isolated bacterial colonies and the results of motility, endospore, catalase and sugar fermentation test are summarized in Table 3. Of the 8 isolates, 1 isolate was Gram-negative and the remaining 7 isolates are Gram-positive. C6 and C7 show cocci shaped and the remaining colonies are rod-shaped bacteria. The motility test showed that the 3 isolates (C1,C2, and C8) were motile whereas the remaining 5 isolates are non-motile growing in the confined stab line. Endospore test revealed that seven isolates were non-endospore-forming bacteria and one (C1) was found to be endospore-forming bacteria. The catalase test study showed that five isolates had catalase-negative results and the remaining three isolates were able to produce bubbles when mixed with hydrogen peroxide. Among eight isolates, two isolates (C3, C7) were heterofermentative and fermented glucose to acid with the change of colour from medium red to yellow and gas production indicated by bubble formation in Durham's tube. Other isolates were found homofermentative and fermented glucose to acid with the change of the colour of the medium from red to yellow.

Based on the morphological, cultural and biochemical characteristics, the isolates were identified as *Lactobacillus* sp., *Escherichia coli, Bacillus* sp., and *Streptococcus* sp. The results from the present study were compared with the study of Ashwani and Dinesh[16], they isolate different strains of lactic acid bacteria from dairy samples (curd and milk) and all the strains were non-motile. In our study, four isolates were found to be motile and the remaining isolates are non-motile.

Screening for biosurfactant production

After 7 days of incubation, the mineral salt medium was observed for bacterial growth. The bacterial growth is indicated by the presence of turbidity in all the conical flasks. Then the cultures were analysed for the presence and absence of biosurfactant by hemolytic assay followed by oil spreading and drop collapse test.

Preliminary characterization

Hemolytic activity

Six colonies were positive for hemolysis among that some showed the maximum hemolytic activity of

3.05 cm. S1 and *E. coli* were not showed the zone of hemolysis. The minimal zone of hemolysis was observed in B1. In some studies, bacterial strains with positive hemolytic activity were found negative for biosurfactant production (Table 4). Hence, not all biosurfactants had hemolytic activity and compounds other than biosurfactants may cause hemolysis. Thus, the present investigation includes a drop collapse test, oil spreading assay and emulsification to confirm biosurfactant production.

Drop collapse test

In this experiment, cell-free broth was used as the biosurfactant source. Distilled water did not collapse on the oily surface of the well and appeared as a bead. However, the detergent solution collapsed in 1 minute, which is reported in the previous study by Jain *et al.*[18]. Similarly, the result of our experiment indicates that the oil surface collapsed within a few seconds hence it concluded that isolated strains were positive for biosurfactant production. Some isolates showed a drop collapse activity which produced extracellular biosurfactant. Nevertheless, some isolates did not collapse the oil surface they did not produce the biosurfactant since the result was negative. Fig. 1 demonstrates that some microbes gave flat droplets but some gave negative resultsbecause they have high cell hydrophobicity but they not produce extracellular biosurfactant.

Table 4 — Hemolytic and emulsification activity of the isolated bacterial colonies

Bacterial strains	Hemolytic activity	Zone of hemolysis (cm)	Emulsification activity
Bacillus sp. (B1)	+	1	++
Bacillus sp. (B2)	+	0.7	+
Lactobacillus sp. (L1)	+	0.5	++
Lactobacillus sp. (L2)	+	3.05	+++
Lactobacillus sp. (L3)	+	2	++
Streptococcus sp. (S1)	_	_	NE
Streptococcus sp. (S2)	+	1.5	NE
Escherichia coli	_	_	NE

+ = Presence of activity, - = Absence of activity, NE- no emulsification, ++ - emulsification activity 2 to 2.9, +++ - emulsification activity >3

Oil spreading test

Drop collapse assay and oil spreading test gave similar positive isolates. Fig. 2 shows these results confirmed the presence and absence of surface active compounds in the cell-free culture broth. The area of oil displacement is directly proportional to the concentration of the biosurfactant in the solution in an oil-spreading assay. However, in this study, there was no quantitative study conducted for analysis of biosurfactant concentration versus oil spreading activity, buta qualitative study was conducted to check the presence of biosurfactants in the cell-free culture

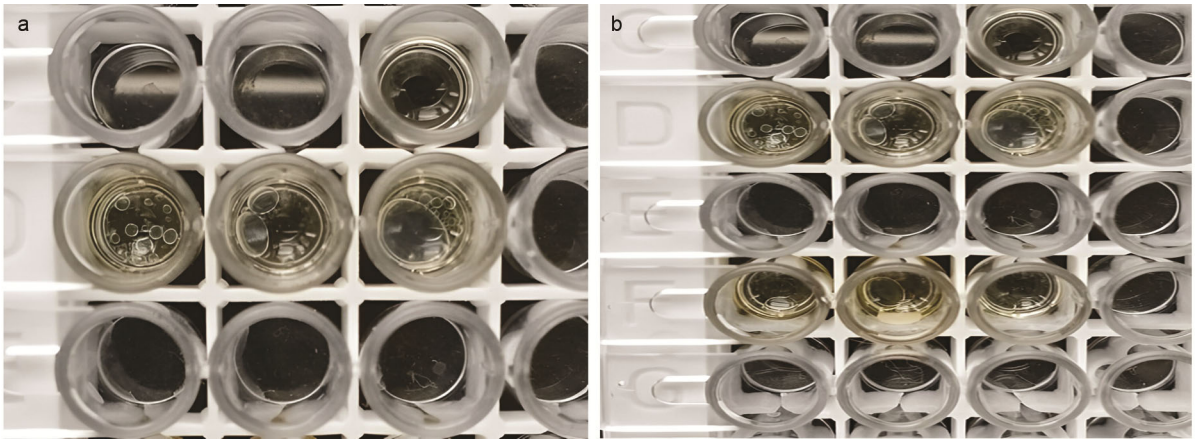

Fig. 1 — Drop collapse test by a) Detergent solution; and b) Extracted biosurfacatnt.

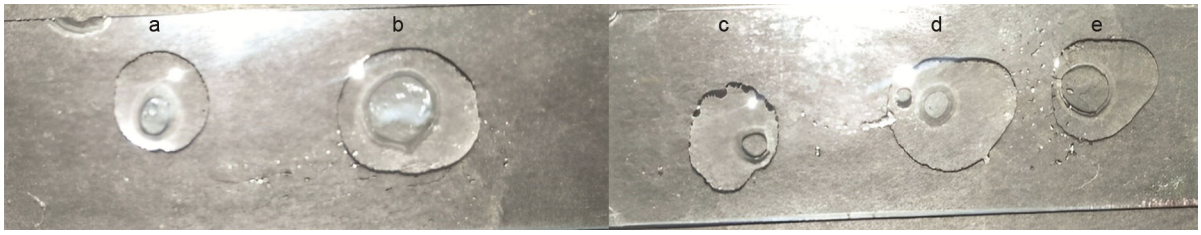

Fig. 2 — Oil spreading zone exhibited by the isolated bacteria of a) B1; b) B2; c) L1; d) L2; and e) L3.

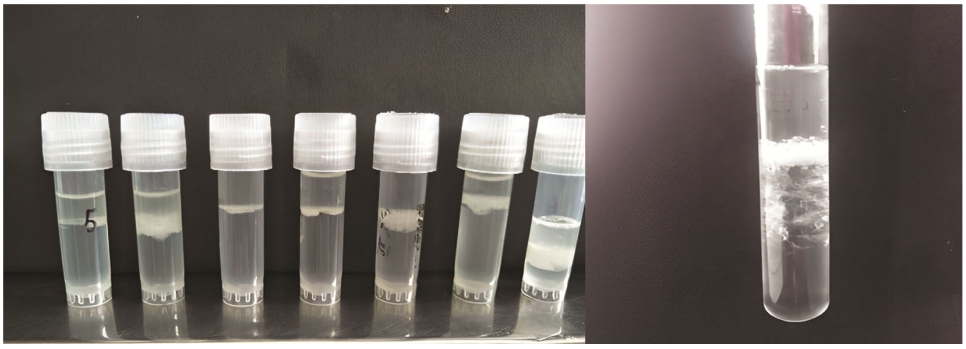

Fig. 3 — Extracted biosurfactants.

broth. Youssef *et al.*[19], mentioned that the oil spreading technique is a better predictor for biosurfactant production than other techniques. Hence, the positive resulted isolates of oil spreading technique were selected for further study.

Complementary screening

Emulsification activity

The results observed in this experiment reveal that except for three bacterial isolates remaining five had positive for emulsification activity (Table 4). Based on the presence of biosurfactants, the result was differentiated which is mentioned in +,++, and +++. The *E.coli, Streptococcus* sp., and *Lactobacillus* sp., (L3) exhibit no emulsification activity. Both *Bacillus* sp.,(B1, B2) have excellent surface activity. Based on the result, an isolate L2 was a biosurfactant-producing bacterium which showed stronger emulsifying activities among other isolates. According to Willumsen and Karlson[20], a criterion used for selecting biosurfactant producers is the ability of maintains at least 50% of the original emulsion volume after 24 h. In which L2 isolate maintains the emulsion volume after 24 h since select L3 isolate for biosurfactant production undoubtedly.

Antibacterial activity of the isolated biosurfactants

Biosurfactant exhibits strong antibacterial properties, making them suitable for infection control applications[21]. Fig. 3 shows that precipitated pellets are regarded as biosurfactants. Based on the emulsification activities, we selected five isolates such as B1, B2, L1, L2, and L3 for the antibacterial activity test (Table 5). The antibiotic sensitivity pattern analysis of different concentrations of biosurfactants was tested against the bacterial isolates and the results were tabulated in Table 6. Rayeni and Nezhad[17] study mentioned that the biosurfactants had showed better effects on sensitivity pattern against indicator strains. In the present study, isolated biosurfactants were found more effective against various pathogenic and non-pathogenic microorganisms that present in milk samples. Jessica *et al.*, and Nalini *et al.*[22,23], has said that surfactin produced by *B. subtilis* inhibits the growth of pathogenic organisms.

Table 5 — Antibacterial activity of different concentrations of biosurfactants against isolated bacteria

Isolates	Biosurfactants	Zone of inhibition (mm)			
		5 mg/mL	10 mg/mL	15 mg/mL	20 mg/mL
Bacillus sp. (B1)	Bs-1	1	1	7	11
Bacillus sp. (B2)	Bs-2	4	6	9	9
Lactobacillus sp. (L1)	Bs-3	3	5	11	13
Lactobacillus sp. (L2)	Bs-4	3	7	15	19
Lactobacillus sp. (L3)	Bs-5	4	5	12	15

Table 6 — Antibacterial activity of the standard drugs against isolated bacteria

Isolates	Zone of inhibition (mm)		
	Gentamicin	Ampicillin	Amoxicillin
Bacillus sp. (B1)	15	9	8
Bacillus sp. (B2)	14	7	7
Lactobacillus sp. (L1)	14	7	6
Lactobacillus sp. (L2)	21	10	11
Lactobacillus sp. (L3)	19	9	10

The purified biosurfactants from bacterial isolates showed antibacterial activity against three isolates of *Lactobacillus* sp. and two *Bacillus* sp. (Table 6). Higher concentrations of biosurfactant led to increase the zone of inhibition. The maximum zone of inhibition was observed at 20 mg/mL biosurfactant concentration which gave growth inhibition in the range of 19 mm diameter. Fig. 4 shows that produced biosurfactants mostly inhibits the growth of *Lactobacillus* species than other isolates. The standard

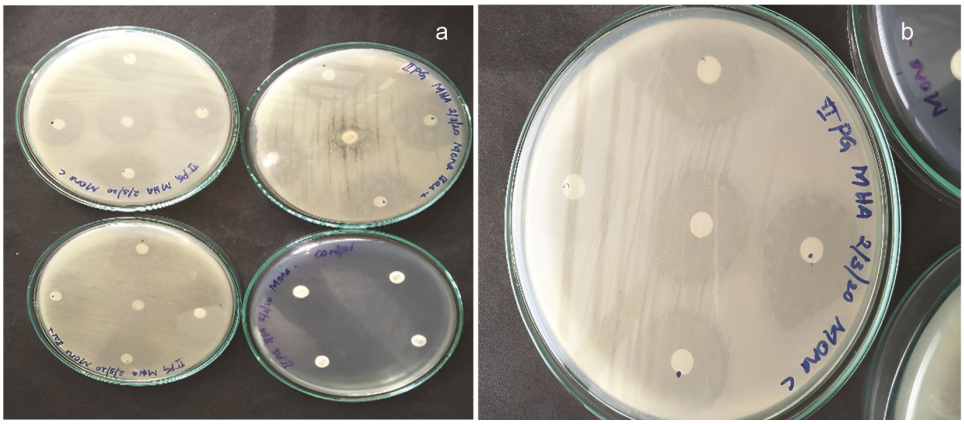

Fig. 4 — Zone of inhibition by the extracted biosurfactants, a) Zone of clearance by *Bacillus* sp.; and b) Zone of clearance by *Lactobacillus* sp.

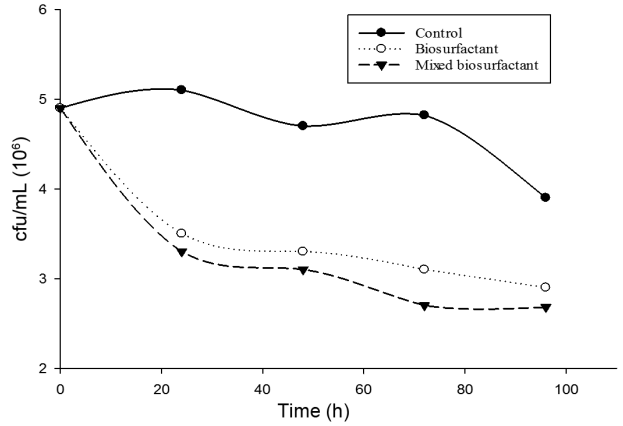

Fig. 5 — Total aerobic bacterial load in raw milk and biosurfactant added milk.

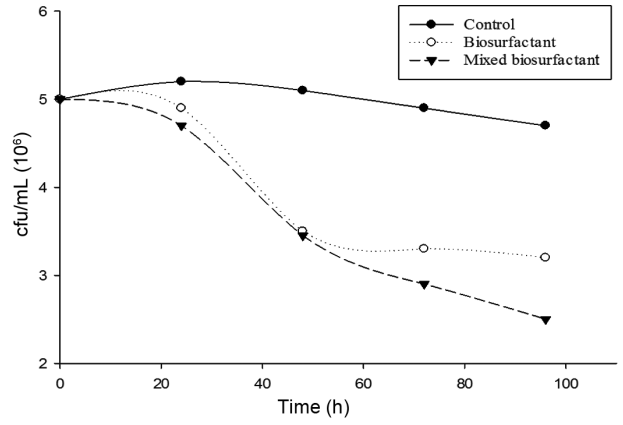

Fig. 6 — Lactic acid bacterial load in raw milk and biosurfactant added milk.

drug gentamicin had showed higher antibacterial activity than other standard drugs (Table 6).

Effect of biosurfactants on microflora of raw milk

Decrease of aerobic bacterial and lactic acid bacterial load of milk was shown in Fig. 5 and 6. Aerobic bacterial load increased in control milk

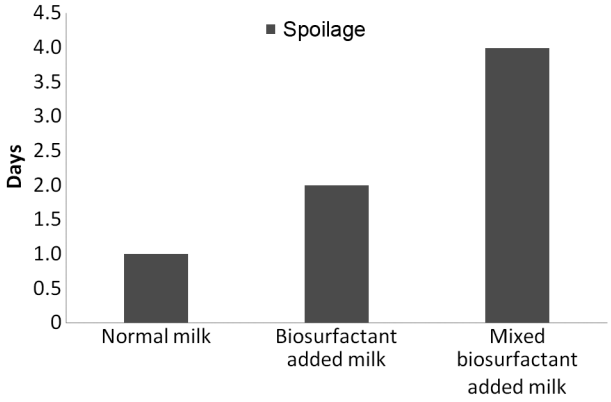

Fig. 7 — Graph showing the shelf life of biosurfactant added and normal milk samples.

sample after 24 h and it is decreased after 72 h for the reason of shortage of nutrient in milk. Milk spoils due to lactic acid production as the result of lactose conversion into glucose and galactose. Lactic acid creates caesin and then forms a curd that can quickly curdle the milk within 24 h. So, the higher amount of bacterial load in control sample causes the spoilage of milk within a day (Fig. 7). But the addition of biosurfactant slightly decreases the aerobic and lactic acid bacterial load hence it prevents the milk from spoilage. Fig. 5 and 6 revealed that the mixed biosurfactant had greater ability to decrease the microbic load in raw milk than separately added biosurfactants. This reduction of bacterial load exhibits the antibacterial activity of biosurfactants. In biosurfactant added milk samples, curdling happened after 2-3 days but in normal milk samples it happened in one day. In a previous study by Sharma *et al.*[24], the biosurfactant coated vegetables showed increased shelf life than the uncoated vegetables. Likewise, in our study the biosurfactant added milk samples showed increased shelf life than non-added milk

samples. The biosurfactants prevent thelactic acid production that increases the shelf life of raw milk which caused by inhibiting the growth of spoilage causing microbes. This study shows that produced biosurfactants had better shelf life properties which potentially increase the shelf life of raw milk.

Conclusion

The biosurfactant extracted from the cow's milk increasing the shelf life of the milk for some time. This organism has the capacity to control the conversion of components in the milk. This biosurfactant can be used as a preservative to increase the shelf life of milk up to prolonged periods. In future modification of genes in biosurfactant producing microbes can leads to preserve milk without chemical agents. All of the assay results confirming the best effect of isolated biosurfactant in least quantity. This could be encouraging the industrial development of biosurfactant with microbes in a dread investment. Concluding this article with information of, preservative production for increasing the shelf life of milk will supports the future development.

Conflicts of interest

The authors declare no conflicts of interest in the work submitted.

References

1 ErasMunoz E, Farre A, Sanchez A, Font X and Gea T, Microbial biosurfactants: A review of recent environmental applications, *Bioengineered*, 2022, **13**(5), 12365-12391.

2 Kumar A and Chordia N, Role of microbes in dairy industry, *NutrFood SciInt J*, 2017, **3**(3), 1-3.

3 Yuan H, Han S, Zhang S, Xue Y, Zhang Y, *et al.*, Microbial properties of raw milk throughout the year and their relationship to quality parameters, *Foods*, 2022, **11**, 3077.

4 Anderson M, Hinds P, Hurditt S, Miller P, McGrowder D, *et al.*, the microbial content of unexpired pasteurized milk from selected supermarkets in a developing country, *Asian Pac J Trop Med*, 2011, **1**(3), 205-211.

5 Augustin M, Majeste P M, Hippolyte M T and Leopold T N, Effect of biosurfactants extracted from a locally fermented milk on its shelf life, *J Adv Biol Biotechnol*, 2021, **3**(1), 12-22.

6 Fardami A Y, Kawo A H, Yahaya S, Lawal I, Abubakar A S, *et al.*, A review on biosurfactant properties, production and producing microorganisms, *J Biochem Microbiol Biotechnol*, 2022, **10**(1), 5-12.

7 Sanjana M C, Ramachandra B and Prabha R, Isolation of biosurfactants producing *Bacillus* spp. From milk samples, *Pharm Innov J*, 2022, **8**(4), 690-692

8 Araujo J, Monteiro J, Silva D, Alencar A, Silva K, *et al.*, Surface active compounds produced by microorganisms, *Antibiotics*, 2022, **11**, 1106.

9 Saharan B S, Sahu R K and Sharma D, A review on biosurfactants: fermentation, current developments and perspectives, *J Genet Eng Biotechnol*, 2011, **2011**, 1-14.

10 Schultz J and Rosado A S, Extreme environments: A source of biosurfactants for biotechnological aplications, *Extremophiles*, 2020, **24**, 189-206.

11 Kumar A, Singh S K, Kant C, Verma H, Kumar D, *et al.*, Microbial biosurfactant: A new frontier for sustainable agriculture and pharmaceutical industries, *Antioxidants*, 2021, **10**, 1472.

12 Muthusamy K, Gopalakrishnan S, Ravi T K and Sivachidambaram, Biosurfactants: Properties, commercial production and application, *Curr Sci*, 2008, **94**(6), 736-747.

13 De Oliveira E M, Sales V HG, Andrade M S, ZilliJ E, Borges W L, *et al*., Isolation and characterization of biosurfactant producing bacteria from Amapaense Amazon soils, *Int J Microbiol*, 2021, **2021**, 1-11.

14 Naughton P J, Marchant R, Naughton V and Banat I M, Microbial biosurfactants: Current trends and applications in agricultural and biomedical industries, *J Appl Microbiol*, 2019, **127**(1), 12-28.

15 Kumar A and Kumar D, Isolation and characterization of bacteria from dairy samples of solan in Himachal Pradesh for identification of *Lactobacillus* spp, *Int J Pharm Sci Rev Res*, 2014, **25**(2), 110-114.

16 Nayarisseri A, Singh P and Singh SK, Screening, isolation and characterization of biosurfactant producing *Bacillus subtilis* strain ANSKLAB03, *Bioinformation*, 2018, **14**(6), 304-314.

17 Rayeni LT and Nezhad S S, Characterization of biosurfactant produced by probiotic bacteria isolated from human breast milk, *Int J Basic Sci Med*, 2018, **3**(1), 18-24.

18 Jain D K, Collins-Thompson D L, Lee H and Trevors T, A drop-collapsing test for screening surfactant-producing microorganisms, *J Microbiol Methods*, 1991, **13**(4), 271-279.

19 Youssef N H, Duncan K E, Nagle D P and Savage K N, Comparison of methods to detect biosurfactant production by diverse microorganism, *J MicrobiolMethods*, 2004, **56**(3), 339-347.

20 Willumsen P A and Karlson U, Screening of bacteria, isolated from PAH- contaminated soils, for production of biosurfactant and bioemulsifiers, *Biodegradation*, 1997, **7**(5), 415-423.

21 Sambanthamoorthy K, Feng X, Patel R, Patel S and Paranavitana C, Antimicrobial potential of biosurfactants isolated from lactobacilli against multi drug resistant pathogens, *BMC Microbiol*, 2022, **10**, 197.

22 Jessica M, Yuliani H, Perdani M S, Savitri I, Meilani M, *et al.*, Antimicrobial activity of biosurfactant derived fom *Bacillus subtilis* C19, *Energy Procedia*, 2022, **153**(2), 274-278.

23 Nalini R, Sanjana M C, Yadav K S, Malashree L and Prabha R, Bacterial biosurfactants - A boon to dairy industry, *Int J Curr Microbiol Appl Sci*, 2022, **10**(7), 515-519.

24 Sharma V, Garg M, Talukdar D, Thakur P, Henkel M, *et al.*, Preservation of microbial spoilage of food by biosurfactant-based coating, *Asian J Pharm Clin Res*, 2018, **11**(2), 98.