Isolation, purification and characterization of bioactive surfactant from the probiotic bacterium *Lactobacillus casei* NM512 isolated from infant microbiota

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Background
Lactobacillus casei NM512 was previously isolated from the feces of breast-fed infants and has great potential for probiotic treatment of diseases that are based on immune deregulation. These strains fulfill the main criteria required for safe human consumption and could therefore be useful in pharmaceutical and therapeutic preparations. However, further studies are needed to evaluate and decipher the in vivo activities of the isolated strains with regard to their immunomodulatory function.
Objective
This work aims to isolate, identify and characterize the bioactive surfactant component from the probiotic bacterium *L. casei* NM512.
Materials and methods
The isolation of the surfactant from *L. casei* NM512 was performed after incubation the strain for 72 h at 37°C with shaking at 120 rpm. The surfactant extracted from cells by acid precipitation. A combination of different methods (TLC, FT-IR, NMR, and GC mass) was used to analyze the composition of the isolated surfactant. The surfactant was also investigated for its antimicrobial activity using the well agar diffusion method, antioxidant ability targeting the DPPH free radical, Cytotoxicity ability using MTT assay, and anti- inflammatory activity was done by determination the inhibition of nitric oxide (NO) production in RAW 264.7 macrophages.
Results and conclusion
The obtained data show that the surfactant is a mixture of carbohydrates, lipids and proteins with glycolipopeptide nature that expressed remarkable antibacterial and antioxidant activities. Furthermore the isolated surfactant showed safety by keeping the cell viability and anti-inflammatory activity. Here we introduce a novel biosurfactant for medical and pharmaceutical purposes.

Keywords:
antimicrobial, antioxidant, Biosufactant, L. casei NM512, probiotics

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Introduction

The strain *Latobacillus casei* NM512 was previously isolated from breast-fed infants stool microbiota [1] and its partial 16S rRNA gene was submitted to the GenBank under accession number KC481685. The selection of this strain was according to its probiotic and promising immunomodulatory properties. The strain *L. casei* NM512 induced IL-6 and TNF in MNCs isolated from healthy human donors also the release of IL-12, IL-1b, IL-6 and CXCL-8. Moreover, the strain *L. casei* NM512 strongly activated Toll-like receptor 2, thus lipopeptides and Muramyl Dipeptide (MDP) located in the cell surface were suggested as the bioactive immunomodulatory structure.

Biosurfactants are known as biomolecules that created by different microbes; bacteria, fungi, and, yeasts [2]. Consequently, the biosurfactants are basically diverse according to the microorganism and species even in the strain level [3]. Each biosurfactant contains hydrophilic and hydrophobic moiety and classified according to their chemical nature, molecular weight, or microbial origin. Basically they could be classified into two molecules types the low molecular weight for instance (flavolipids, glycolipids, lipopeptides) and the high molecular weight as (lipopolysaccharides, polysaccharides, lipoproteins) [4]. Research concerning biosurfactants commenced from long time ago but the usage of these biosurfactants has prolonged in recent eras [5,6]. These compounds catch the attention of diverse

manufacturing as they pose benefits related to their physical variety, safety, biodegradability, in addition to their usage in different industries and by-products [7–9].

Lactobacillus species is one of the most important genera of lactic acid bacteria (LAB) [10]. They have long history in dairy and food fermentation in addition many strains of Lactobacillus species showed probiotic properties. Probiotics are living microbial preparations that have beneficial effects on the well-being of the host when administered in adequate amount [11]. Thus, biosurfactant production from probiotic bacteria especially lactobacillus and other members of Lactic acid bacteria became an attractive target in recent years. Their biosurfactant also displayed antimicrobial as well as anti-adhesive activity which competing the colonization of pathogens [12,13].

This study aimed to isolation and characterization biosurfactant from *L. casei* NM512. The isolated biosurfactant was purified, characterized also their antimicrobial and antioxidant activities were evaluated providing a promising applications in probiotic and pharmaceutical preparations.

Materials and methods

Bacterial strains and growth conditions

Lactobacillus strain was grown on MRS agar at 37°C (Difco, Franklin Lakes, NJ, USA). The indicator strains were cultivated as follows: *Staphylococcus aureus* (ATCC 29213) on BHI agar; *Escherichia coli* (ATCC 25922) on LB agar, *Helicobacter pylori* on blood agar, *Pseudomonas aeruginosa* (ATCC 27953) on LB agar and *Candida albicans* on nutrient agar

Isolation of biosurfactant

Method as described previously [14] was used to isolate the biosurfactant from the bacterial strain, whereas 600 ml of MRS broth in 1 L Erlenmeyer flask were inoculated by 1 % of an overnight culture *L. casei* NM512 followed by incubation for 72 h at 37°C on a rotatory shaker at 120 rpm. Then the cells were harvested by centrifugation (10,000×*g* for 5 min at 10°C), washed twice in demineralized water, and suspended in 100 ml of phosphate-buffered saline solution (PBS) pH 7.0. Cell suspensions were incubated at room temperature for 2 h with gentle stirring to release the biosurfactant then followed by centrifugation. The supernatant was dried in an oven at 70°C and the biomolecules were extracted by acid precipitation as described by Van Hoogmoed *et al.* [15]. The acidified samples were incubated at 4°C for 2 h, and the precipitates were collected by centrifugation (10,000×*g* for 15 min at 4°C) and washed twice with acidic water (pH 2.0). The precipitates were dissolved in distilled water and adjusted to pH 7.0 using 1 M NaOH [16].

Chemical characterization of the isolated biosurfactants

The characterization of biosurfactant was achieved at Central Laboratories Network, National Research Centre, Giza, Egypt (https://www.nrc.sci.eg/centrel-labs/).

Thin layer chromatography (TLC)

The composition of the cell-bound partially purified biosurfactants was determined by TLC followed by post chromatographic detection. Briefly, 4 ml of PBS extract was extracted twice with ethyl acetate 1:1.25 (Rankem, India). Upper phase was extracted two times with ethyl acetate and the ethyl acetate was allowed to evaporate at room temperature [17,18]. 1 ml aliquot of biosurfactants extract was concentrated and separated on a precoated silica gel plate (Merck, India) [19]. Chloroform, methanol and water in the ratio of 70:10:0.5 was used as developing solvent system with different color developing reagents. Two such reagents like, ninhydrin reagent (0.5 g ninhydrin in 100 ml anhydrous acetone) was used for lipopeptide biosurfactant detection as red spots and anthrone reagent (1 g anthrone in 5 ml sulfuric acid mixed with 95 ml ethanol) to detect glycolipid biosurfactant as yellow spots [20].

Fourier-transform infrared spectroscopy (FT-IR)

The infrared spectra (with wave numbers spanning from 4000 to 400 cm^{-1}) were recorded in a Shimadzu FT-IR-8400 spectrometer using 2 mg of the extracted biosurfactant combined with 200 mg KBr (Spectroscopic Grade). The data acquired represented an average of 50 scans across the whole range [21].

Nuclear magnetic resonance (NMR) spectroscopy

The 1–3 mg biosurfactant was dissolved in 100% DMSO and ^{13}CNMR analysis was carried out using a Bruker Av II-400 spectrometer. Both proton and carbon NMR chemical shifts were stated in ppm relative to the solvent shift as chemical standard. Peaks were compared and predicted with the data reported previously [22].

Gas chromatography–mass spectrometry (GC-MS)

Fatty acids were analyzed by GC-MS after conversion to their methyl esters derivatives according to method

described by [23] using a Shimadzu GC–MS model QP 5050 A (Shimadzu, Kyoto, Japan) equipped with a PTE-5-Supelco column (30 m×0.25 mm ID, 0.25 μm film) and employing He as the carrier gas at 0.8 ml min^{-1}, split of 20 and 50 kPa pressure.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity

The scavenging activity for DPPH free radical was measured according to Zhao *et al*. (2006) [24] with some modifications. Sample solutions of the bio-surfactant (100 μL) at different concentrations (2.5, 5.0, 10.0, 20.0, and 40.0 mg/ml) were mixed with 900 μL of 0.1mM DPPH solution in methanol. The mixture was shaken vigorously and allowed to reach a steady state for 30 min in dark at temperature 37°C. Decolourization of DPPH was determined by measuring the absorbance at 517 nm, and the DPPH radical scavenging was calculated according to the following equation: % Scavenging activity= (A1-A2/A1) × 100

Where $\mathbf{A_1}$ was the absorbance of the DPPH solution without sample and $\mathbf{A_2}$ was the absorbance of DPPH with the sample. Ascorbic acid was taken as the standard. All the tests were performed in triplicate.

Cytotoxicity activity (MTT assay)

MTT assay is colorimetric assay based on enzymatic activity of intracellular mitochondrial succinate dehydrogenase enzymes in metabolically active cells to reduce the yellow water soluble substrate MTT into an insoluble purple formazan. Formazan is an insoluble blue crystalline product that can be dissolved with an appropriate detergent and measured spectrophotometrically. The amount of formed formazan is directly proportional to the number of viable cells [25].

The cytotoxicity activity in the present study is based essentially on previous report [25] with some modification. Briefly, MDA-MB-231 cells (8000 cells/well) were seeded onto 96-well plates in a total volume of 200 μl and left overnight to form a semi-confluent monolayer. Cell monolayers were treated in quadrate with test samples of a concentration 100 μg/ml for an exposure time of 48 h. Doxorubicin hydrochloride was used as reference cytotoxic drugs. At the end of exposure, 30 μl/well of MTT solution in DPBS (5 mg/ml) was added to all wells and left to incubate for 90 min. The formation of formazan crystals was visually confirmed using phase contract inverted microscope (Olympus CK2, Japan). DMSO (100 μl/well) was added to dissolve the formazan crystals with shaking for 10 min after which the absorbance was read at 492 nm against blank(no cells) on a Tristar LB2 microplate reader (Berthold, Germany). The percentage of viability was calculated using the following formula: % viability=×100

Whereas, At: Absorbance of treated cells, Ab: Absorbance of blank (medium only without cells), Ac: Absorbance of control. IC_{50} values were calculated using the dose response curve fit to non-linear regression correlation using Graph Pad Prism V6.0 software (GraphPad Inc., San Diego, USA).

In vitro anti-inflammatory by measurement nitric oxide (NO) production

RAW 264.7 cells 0.5 × 106 cells/mL were seeded into 96-well plates and incubated for overnight. At the next day, non-induced triplicate wells received medium with the sample vehicle (DMSO, 0.1%, by volume). Inflammation group of triplicate wells received the inducer of inflammation (lipopolysaccharide (LPS) at a concentration 100 ng/mL in complete culture media). Preliminary screening was performed on biosurfactant using a single concentration of samples (100 μg/ml) dissolved in DMSO and diluted into culture media containing LPS (Final concentration of DMSO=0.1%v/v. Indomethacin (INDO, 0.25 mM) was used as an anti-inflammatory positive control. After 24 h of incubation, Griess reagent was used to determine nitric oxide (NO) in all wells. Equal volumes of culture supernatants and Griess reagent (1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine-HCl) were mixed and incubated at room temperature for 10 min to form the colored diazonium salt and read at absorbance of 540 nm on a Tristar2 lb ^{TM} microplate reader (Berthold, Germany). NO inhibition % of test biosurfactant was calculated relative to the LPS-induced inflammation group (% NO inhibition of LPS+=zero). For IC50 determination, the biosurfactant was diluted into culture media as described above and pipetted onto triplicate wells to receive increasing concentrations (6.25, 12.5, 25.0, 50.0 and100.0 μg/mL) of the bios with final concentration of DMSO being 0.1% v/v. GraphPad Prism V6 (San Diego, USA) was used to calculate the 50% inhibitory concentration of biosurfactant using non-linear regression curve fit.

Antimicrobial activity assay

The antibacterial activity of partly purified biosurfactants was investigated against Gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria (*Escherichia coli, Helicobacter pylori* and *Pseudomonas aeruginosa*) also tested against fungus

Candida albicans. The identified pathogenic bacteria and fungus were obtained from the Microbiology Laboratory of eco-friendly compound, National Research Center, Giza, Egypt. The antibacterial activity was evaluated by the agar well diffusion method [26]. 200 microliters of the test bacteria (10^7 CFU/mL) were spread on NA medium to test their antibacterial activity. After 2 hours at 37°C incubation, a well (7 mm) was picked and filled with 1 µg biosurfactant (50 µl of 20 µg / ml concentration). As a negative control, sterile distilled water was put into the well. To observe the zone of inhibition, the plates were incubated at 30°C for 24 hours. The experiment was carried out in triplicate.

Results and discussion

Isolation of biosurfactant

The surfactant was isolated from probiotic strain *L. casei* NM512 as described in the Methods section. The biosurfactant production yields were ranging from 2 to 10 g L^{-1} according to the growth condition and shaking during the isolation. This is in agreement with the observations by other researchers that indicated the yields of the surfactant were dependent on the growth media, conditions and temperature [16,25,26].

Characterization the chemical composition of the isolated biosurfactant

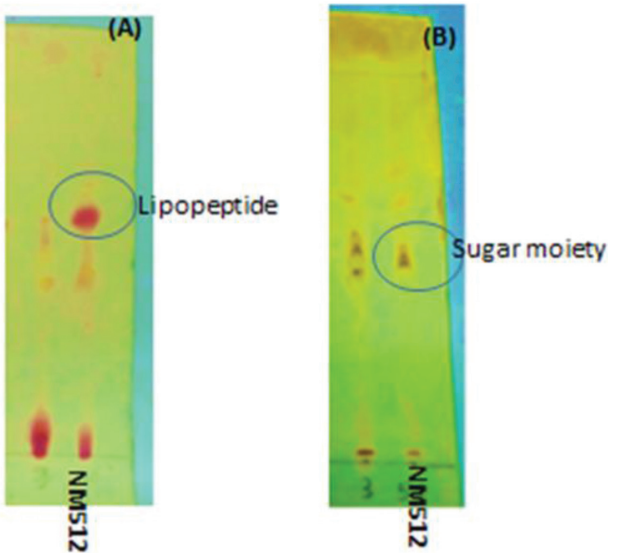
To accurate determination of the chemical composition of the isolated surfactant, the use of combination of different methods as (TLC, FTIR, ^{1}H NMR, ^{13}C NMR and GC-Mass) is needed.

Thin-layer chromatography (TLC)

Thin layer chromatography is widely used for the analysis of surfactants especially both for the characterization of pure surfactants and for the detection of surfactants in complex mixtures due to certain advantageous features such as (i) open and disposable nature of TLC plate (ii) wider choice of stationary and mobile phases (iii) minimal sample cleanup and (iv) reduced need of modern laboratory facilities. So, TLC of the surfactant isolated from *L. casei* NM512 showed red and yellow spots as indicated in Fig. 1a red spot appeared as a result of using the development reagent ninhydrin, which confirm the presence of the lipopeptide fraction acting the hydrophobic part. While in the Fig. 1b yellow spot appeared as a result of using the development reagent anthrone confirming the carbohydrate moiety acting the hydrophilic part.

The structures of the isolated products were elucidated by spectroscopic spectral data (FTIR, ^{1}H NMR, ^{13}C NMR and GC-Mass).

Figure 1



Thin-layer chromatography of the biosurfactant isolated from *L. casei* NM512. Two developing reagents were used (A) Ninhydrin for detection the lipoprotein as red spot, and (B) anthrone for detection glycolipid as yellow spot.

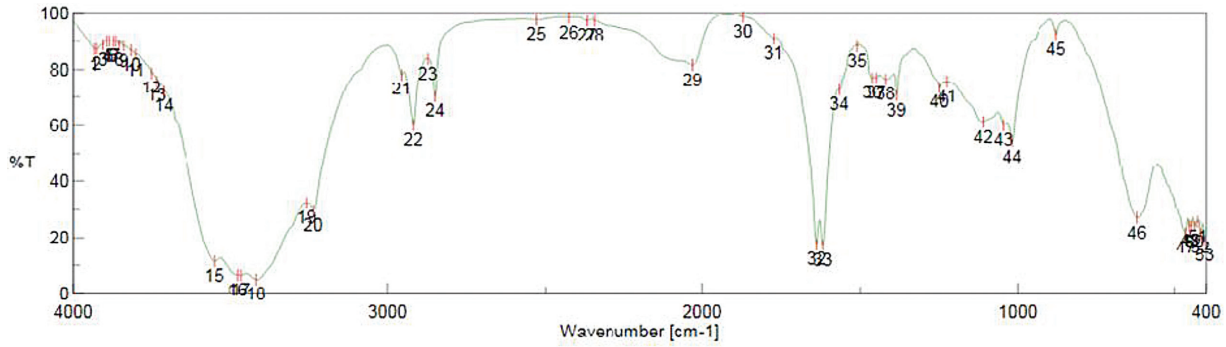
Fourier transform InfraRed (FTIR) analysis

FTIR was implemented to define the molecular components and functional groups of the surfactant isolated from *L. casei* NM512. The spectral analysis by FTIR is shown in Fig. 2 and Table 1. In particular, signals attributable to NH, NH_2, OH, C=O, and CH aliphatic groups are present in IR spectrum. So, the IR of the isolated product exhibited absorption bands in the regions 3000–3600 cm^{-1} corresponding to OH and NH groups. Absorption band at 1509 cm^{-1} corresponding to groups N-H in protein and aliphatic -CH stretching bands (CH_3, CH_2, and CH) appeared in the regions 2850–2960 cm^{-1} indicating the presence of aliphatic side chain might be related to the glycopeptide fraction. Also, the IR spectrum showed the signal of ester carbonyl group of sugar acetate moiety at 1775 cm^{-1} and amide carbonyl group of the peptide at 1640 cm^{-1}.The above data approve that the isolated surfactant has a complex structure of glycolipoproteins. Similar peaks were observed in other *Lactobacilli* strains as *L. casei* MRTL3 [4] and *L. helveticus* [27].

Characterization by NMR

The surfactant was further analysis by ^{1}H NMR and ^{13}C NMR and the results are shown in Figs. 3 and 4 in addition to Tables 2 and 3. ^{1}H NMR results showed the occurrence of -CH3-(CH_2) - at (0.896–0.926 ppm), -(CH_2)–at (1.270 ppm), -(CH_2–OH)– at (3.549 ppm), 1-H (sugar) at (4.224 ppm) . Also, the ^{1}H NMR spectrum showed the anomeric proton of the sugar moiety as a doublet at

Figure 2



The spectral analysis of the isolated surfactant from *L. casei* 512 by FTIR.

Table 1 The function groups detected by FTIR of surfactant isolated from *L. casei* NM512

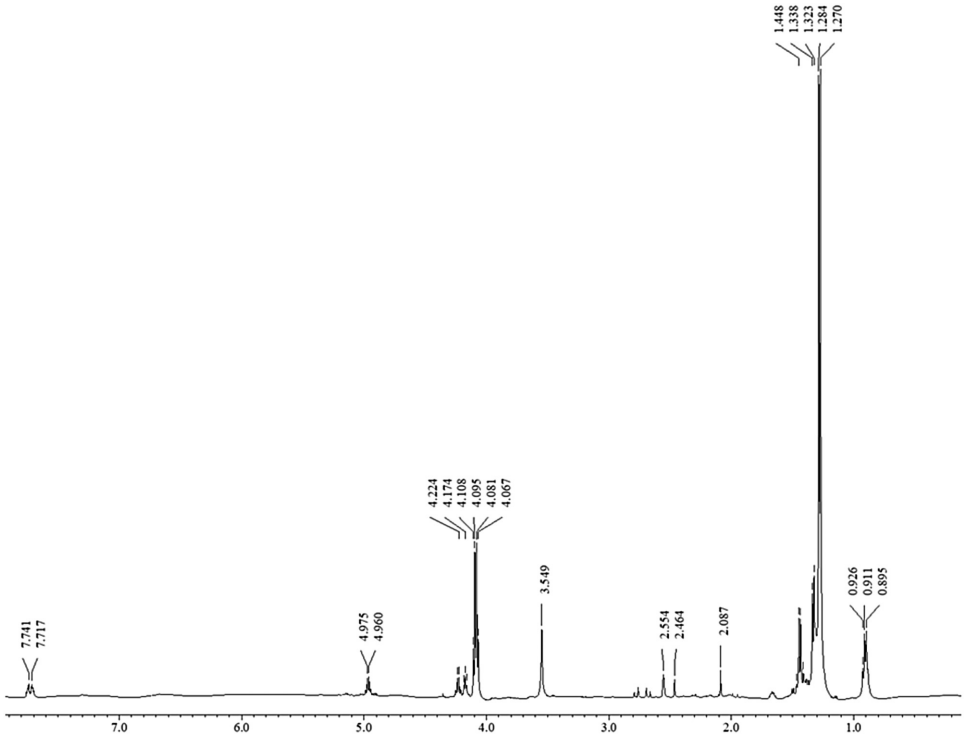
Absorbance range (cm^{-1})	Functional groups detected
3000–3600	OH stretching, typical polysaccharides
2850–2960	C-H (stretching) groups CH_2 and CH_3
1775, 1640, 1615	C=O (stretching of carbonyl group)
1509	Groups N-H in proteins
1400–1460	C=H stretching
1000–1300	C-O sugar stretching
675–1000	C-H bending

δ 4.96 ppm. The other protons of the sugar ring resonated at δ 2.00–4.22 ppm. The results from ^{13}C NMR indicated C-1 at 128.63 ppm, C-2 and C-3 at 60.74 ppm, C-4 at 69.63 ppm, C-5 and OCH3 at 55.48 ppm, and CO at 166.73–176.87. The results propose that surfactant isolated from *L. casei* NM512 is glycolipopeptide. Our results are matching the results obtained with other strains as *L. pentosus* NCIM 2912 [28] and *Lactococcus lactis* CECT 4434 [29].

Characterization by GC-mass

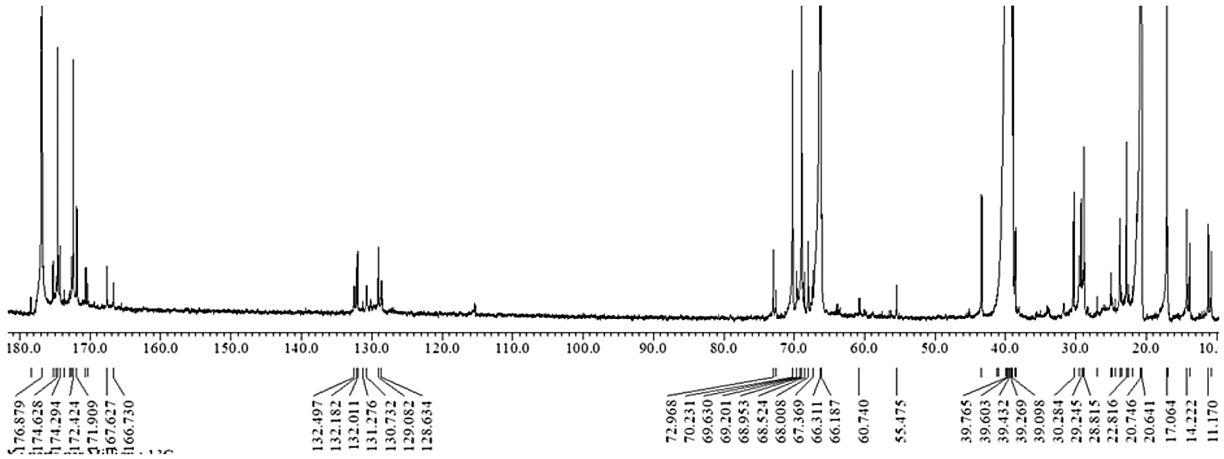
GC-mass results for surfactant isolated from *L. casei* NM512 are presented in Table 4 and Fig. 5 showed detection of four peaks with highest area sum % (9.64, 22.13, 22.84, 23.74‘) with molecular formula of

Figure 3



^{1}H NMR Spectra of the biosurfactant isolated from *L. casei* NM512.

Figure 4



^{13}C NMR Spectra of the biosurfactant isolated from *L. casei* NM512.

Table 2 Chemical shift assignment of biosurfactant isolated from *L. casei* NM512 (^{1}H NMR)

Assignment(s)	Chemical shift ^{1}H NMR (ppm)
CH_3–(CH_2)–	0.895–0.926
–(CH_2)–	1.270,1284
–CH_2OH	3.549
1-H (sugar)	4.224

Table 3 Chemical shift assignment of surfactant isolated from *L. casei* NM512 (^{13}C NMR)

Assignment(s)	Chemical shift ^{13}C NMR (ppm)
C-1	128.63
C-2	60.74
C-3	60.74
C-4	69.63
C-5	55.48
OCH_3	55.48
COOH	166.73–176.87

$C_{16}H_{32}O_2$ on RT 4.916 named Hexadecanoic acid, $C_{18}H_{32}O_2$ on RT 8.784 named 16-methyl-Heptadecanoic acid, $C_{16}H_{32}O_2$ on RT 9.746 named 14-methyl-Pentadecanoic acid, and $C_{18}H_{32}O_2$ on RT 12.338 named Stearic acid. The results are in similar patterns with others obtained from surfactant isolated from Lactic acid bacteria [30].

DPPH radical scavenging ability

To do assessment of the antioxidant for the BS (*L. casei* NM512) the DPPH method was used different concentrations (5, 10, 20, 40 mg/ml) were tested as shown in Table 5 the BS demonstrated 86.7% ±0.68 radical scavenging ability at a concentration of 40 mg/ml (Table 5), the antioxidant of the biosurfactants were confirmed by other studies [31].

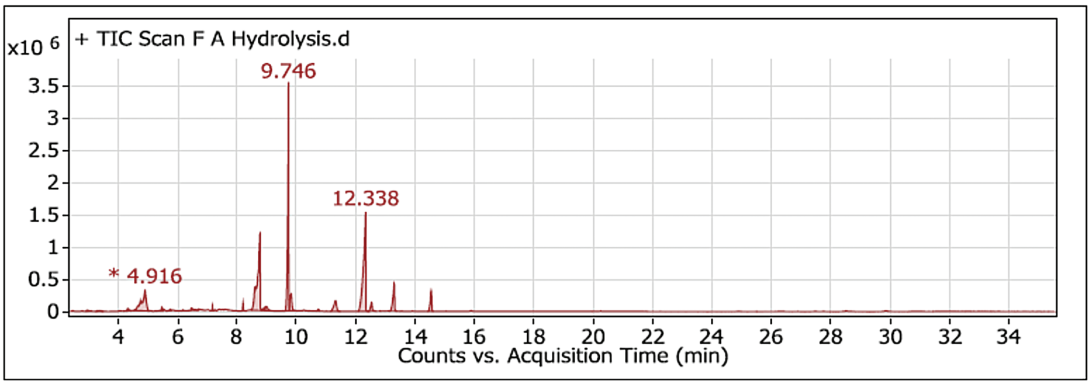
Cytotoxicity assessment

Cytotoxicity assessment was done for the isolated surfactant to address its ability to affect the cell viability. Initial screening of isolated surfactant with concentration 100 µg/ml was done by MTT assay against the proliferation of MDA-MB-231 cancer cell line revealed 79.58 ± 0.19 % viability. This percentage is considering higher than value obtained

Table 4 Fatty acids are present in the surfactant isolated from *L. casei* NM512

Peak	RT	Name	Formula	Area Sum %
1	4.916	Hexadecanoic acid	$C_{16}H_{32}O_2$	9.64
2	8.212	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.52
3	8.784	16-methyl-Heptadecanoic acid	$C_{18}H_{36}O_2$	22.13
4	9.002	(*Z*)-10-pentadecenoic acid	$C_{15}H_{28}O_2$	1.03
5	9.746	14-methyl-Pentadecanoic acid	$C_{16}H_{32}O_2$	22.84
6	9.831	11,12-octadecadienoic acid	$C_{18}H_{32}O_2$	3.73
7	10.747	Heneicosanoic acid	$C_{21}H_{42}O_2$	0.25
8	12.338	Stearic acid	$C_{18}H_{32}O_2$	23.74
9	12.538	(*Z*)-9-Octadecenoic acid	$C_{18}H_{34}O_2$	1.64
10	13.299	(Z,Z)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	4.99
Sum				**90.51**

Figure 5



Total ion chromatogram (TIC) for GC-MS of the surfactant isolated from *L. casei* NM512.

Table 5 Scavenging activity % of surfactant isolated from *L. casei* NM512

Concentration (mg/ml)	% Scavenging activity
40	86.7±0.68
20	85.5±1.41
10	76.3±0.65
5	49.6±1.46
2.5	30.4±0.83
IC50(mg/ml)	3.05

Data are presented as mean±SE.

from surfactant isolated from *L. pentosus* NCIM 2912 by Sharma et al. (2022) [28]. The authors [28] used a higher concentration (1 mg/ml) than what we used in the current study (100 µg/ml) and revealed 90.3 ± 0.1%, 94.3 ± 0.2%, and 99.2 ± 0.43% viability with cell lines; HEK 293, mouse fibroblast, and HEP-2 in turn. The low cytotoxicity is necessary for application the surfactant in medical purposes or food processing.

Anti-inflammatory assessment

Surfactant isolated from *L. casei* NM512 was tested for anti-inflammatory activity to inhibit the Lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophages. Data presented in Fig. 6 revealed that a concentration dependent effect of the extract (12.5-25-50 and 100 ug/ml) inhibited nitric oxide production. Concentration of the extract that inhibits 50% of nitric oxide induced with LPS only was calculated from the non-linear regression curve fit shown above with IC50=95.5 ug/ml. In the other hand, rare anti-inflammatory possessions linked to glycolipid surfactant have been stated as the case of surfactant isolated form *C. bombicola* reduced the expression of TNF-α, COX-2, and IL-6 in RAW 264.7 cells [32].

Antimicrobial activity

The assessment of antimicrobial competency of the isolated biosurfactant from *L. casei* NM512 was performed against five indicator pathogens including yeast, gram positive and gram negative i.e. (*C. albicans, St. aureus, P. aeruginosa, E. coli, H. pylori*) using ager well diffusion test (Fig. 7) this confirmed the antimicrobial action of the biosurfactant. Table 6 shows the Antimicrobial zones of isolated surfactant.

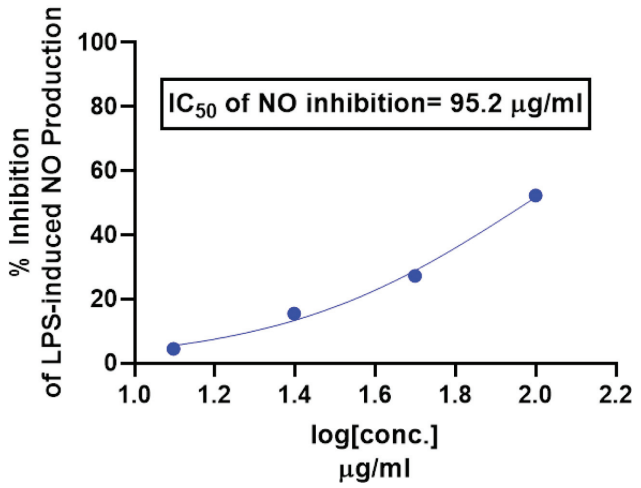
For The Minimum inhibition concentration (MIC) (Table 7) of the isolated surfactant was 12.5 mg ml^{-1} for *H. pylori* and *P. Aeruginosa* and 37.5 mg ml^{-1} for *E. coli, St. aureus* and *C. albicans*.

Previous research showed that biosurfactants (BS) isolated from different microorganisms demonstrated antibacterial activity. Sharma and Saharan (2016) [33] described the antibacterial activity of the biosurfactant isolated from *L. helveticus* against *P. aeruginosa*, *S. typhi*, *Sh. flexneri* and *C. albicans*. While

Table 6 Antimicrobial zones of isolated surfactant isolated from *L. casei* NM512 against the pathogenic indicator strains using an agar well diffusion assay. Diameters of zones of inhibition are presented in mm. (a, b, c) represent three independent experiments

	Inhibition zone (mm)				
	E. coli	*St. aureus*	*C. albicans*	*Ps. aeruginosa*	*H. pylori*
NM512 (a)	31	29	33	30	32
NM512 (b)	32	28	31	29	33
NM512 (c)	30	28	33	30	30

Figure 6



Anti-inflammatory potential of BS isolated from *L. casei* NM512 on RAW 264.7 macrophage model.

Table 7 Minimum Inhibition Concentration of the surfactant isolated from *L. casei* NM512 using different concentrations up to 50 mg

	50 mg	25 mg	10 mg	5 mg	2.5 mg
H. pylori	0.1123	0.4382	0.5447	0.6486	0.8508
E. coli	0.1578	0.4919	0.5129	0.7988	0.8544
St. aureus	0.1598	0.5455	0.8595	0.9324	1.6393
C. albicens	0.1633	0.4904	0.6864	0.9398	1.1492
Ps. aeruginosa	0.2080	0.4359	0.6539	0.8183	1.100
Blank			0.4833		

biosurfactant isolated from *L. gasseri* BC9 exhibited antibacterial activity against methicillin resistant *S. aureus* (MRSA) [34]. De Gregorio et al. (2020) [35] presented the antibacterial activity of BS isolated from vaginal *L. crispatus* BC1 against *Candida* spp. including *C. albicans*.

The growing claim for innovative agents to fight the enlarged global antimicrobial resistance problem thus, several researchers are focusing on the use biosurfactant as antimicrobial agent against resistance pathogens

Conclusion

The current work aimed to isolate and characterize the biosurfactant of the *L. casei* NM512 which approved in our previous work as probiotic candidates with remarkable immuondolatory properties. The isolated biosurfactant was characterized as glycopeptide by using combination of methods including TLC, FTIR, ^{1}H NMR, ^{13}C NMR and GC-Mass. Other investigations approved the remarkable antioxidant activity and antibacterial activity of the biosurfactant isolated against the tested pathogens including yeast (*C. candida*), G+ (*S. aureus*), and G- (*H. pylori*, *E. coli*, *Ps. aeruginosa*). Thus, we introduce novel biosurfactant from the probiotic strain *L. casei* NM512 with promising characteristics for future applications in medical and pharmaceutical sectors.

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Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

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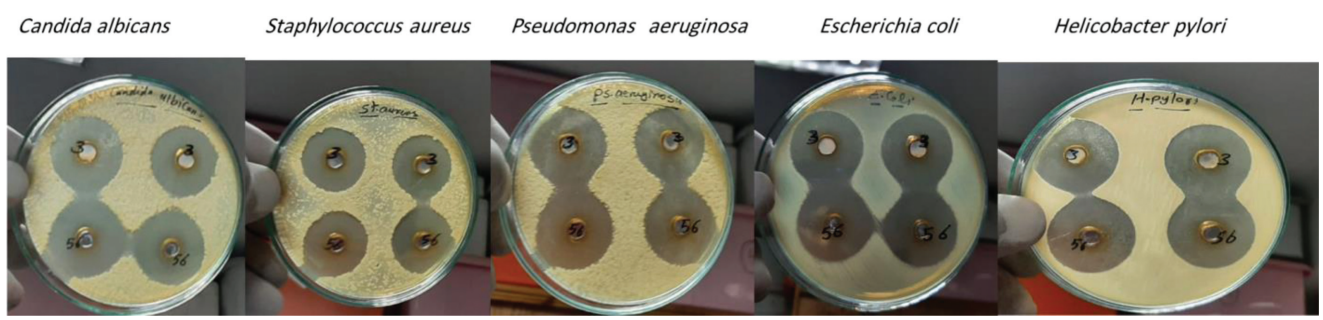
Figure 7



Antimicrobial zones of the surfactant isolated from *L. casei* NM512 (encoded 56) against the pathogenic indicator strains using an agar well diffusion assay.

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