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Research article

Investigating lactic acid bacteria genus *Lactococcus lactis* properties: Antioxidant activity, antibiotic resistance, and antibacterial activity against multidrug-resistant bacteria *Staphylococcus aureus*

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ABSTRACT

Background: Lactic acid bacteria (LAB) are utilized as a starter culture in the manufacturing of fermented dairy items, as a preservative for various food products, and as a probiotic. In our country, some research has been carried out, even if LAB plays a principal role in food preservation and improves the texture and taste of fermented foods, that is why we tried to evaluate their probiotic effect. The objective of this research was to determine the antibacterial activity of *Lactococcus lactis (L. lactis)* against *Staphylococcus aureus* (*S. aureus*) ATCC 29213, investigate their antioxidant activity, and characterize their sensitivity against 18 antibiotics.
Methods: A total of 23 LAB (*L. lactis* subsp. *cremoris, L. lactis* subsp. *Lactis diacetylactis, L. lactis* subsp. *lactis*) were isolated from cow's raw milk. The antibacterial activity was performed using two techniques, competition for nutrients and a technique utilizing components nature, using the disk diffusion method. The sensitivity of the studied LAB to different antibiotics was tested on Man rogosa sharp (MRS) agar using commercial antibiotic disks. All strains of LAB were examined

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for their antioxidant activity. The antioxidant activity of *L. lactis* was tested by 2,2-diphenyl-1 picrylhydrazyl (DPPH).

Results: The results showed that the MRS medium was more adapted than Muller Hinton Agar (MHA) to investigate the antibacterial activity of *L. lactis* against *S. aureus* ATCC 29213. Also, *L. lactis* exhibited a notable degree of antibacterial activity against *S. aureus* ATCC 29213. *L. Lactis* subsp. *Lactis* displayed higher antibacterial activities, followed by *L. lactis* ssp. *lactis biovar. diacetylactis,* and lastly, *L. lactis* ssp. *cremoris* against *S. aureus* ATCC 29213. Lc 26 among all strains of *L. lactis* showed a high potential antibacterial activity reaching 40 ± 3 mm against *S. aureus* ATCC 29213. All strains of *L. lactis* showed a slightly moderate antioxidant activity (10.56 ± 1.28%-26.29 ± 0.05 %). The results of the antibiotic resistance test indicate that all strains of *L. lactis* were resistant to cefotaxime, sulfamethoxazole-trimethoprim, and streptomycin and were sensitive to Ampicillin, Amoxicillin, Penicillin G, Teicoplanin, Vancomycin, Gentamicin 500, Tetracycline, and Chloramphenicol. These test results indicate that this strain falls within the criteria of not posing any harmful effects on human health. The important antibacterial properties recorded for all *L. Lactis* strains were derived from the production of antibacterial active metabolites, such as protein, diacetyl, hydrogen peroxide, and lactic acid, together with the fight for nutrients.

Conclusion: This study suggests that the strains of *L. lactis* could be added as an antibacterial agent against *S. aureus* ATCC 29213 and can provide an important nutritional property for their antioxidant potential.

1. Introduction

Food safety poses a significant public health issue as a result of the occurrence of food-borne illnesses [1–6]. LAB are microorganisms, gram-positive, catalase-negative, acid-tolerant, non-sporulating, and aero-tolerant, naturally found in milk and have long been used for several centuries as protectant agents in fermented food products. They are widely employed in industrial fermentation processes, traditional fermented milk, and as lactic ferments in the dairy industry [7]. LAB constitutes the natural intestinal microflora of humans and most animals [8,9]. LABs have been used in many fermented food products because they are essential in preserving, manufacturing, and producing nutritious foods [10]. Lactic acid bacteria can modify the flavour of dairy products and enhance their quality. Lactococcus are gram-positive cocci belonging to the group of LAB, as well as they are homofermentative [11]. They are used as bio-preservatives in the food industry and as probiotics in the medical field. Lactococcus can inhibit or kill unwanted microorganisms in food, increasing food safety and extending shelf life [12]. The Lactococcus genus comprises various species such as *L. raffinolactis*, *L. plantarum*, *L. garvieae*, *L. piscium*, and *L. lactis*. This latter comprises two subspecies, which are *L. lactis* subsp. *cremoris and L. lactis* subsp. *lactis.* Some strains of this last subspecies are referred to as *L. lactis* subsp. *Lactis bv. Diacetylactis* because they can produce diacetyl and ferment acetoin and citrate [13]. *L. lactis* is the main ingredient of numerous industrial and starter cultures and is generally recognized as safe (GRAS) by the US Food and Drug Administration [14,15]. According to Issa et al., the *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *Lactis* dominate cheese and no-pasteurized raw milk [16].

Contamination of food by pathogenic germs causes food poisoning, which is a major problem for consumers [17]. For this reason, the use of strains that have a protective effect seems to be essential [18].

In the world, food poisoning outbreaks recorded that *Staphylococci* (ST), and dairy are closely linked in their history [19], and declared that ST was considered the causative agent of food poisoning from the consumption of cow's milk [19,20]. *S. aureus* ATCC 29213 belongs to multi-resistant bacteria gram-positive and catalase-positive bacteria [21,22].

The antibiotics used for treating multidrug-resistant *S. aureus* ATCC 29213 pose a challenge as they exhibit multiple antibiotic resistance mechanisms [23]. For this reason, the enzymes endolysin and peptidoglycan hydrolases produced by bacteriophages have been identified as potential alternative antimicrobial agents [24,25]. LAB produces a variety of antimicrobial compounds derived from the fight for space and nutrients. They can be categorized as low molecular weight compounds like hydrogen peroxide, diacetyl, and carbon dioxide, uncharacterized compounds, and high molecular weight compounds like bacteriocins [14,26]. Acetic and lactic acid are important components inhibiting a wide spectrum of microorganisms [27,28]. All antimicrobial compounds can inhibit the development of some undesirable bacteria in food and have been investigated in the fight against most undesirable organisms [29,30]. Scientists are encouraged to search for LAB from natural sources in response to the rising interest in products with nutritional and practical properties [31]. LAB has received a great deal of attention because of the health-enhancing properties of some LAB, called probiotics [32]. Probiotic LAB is generally considered safe and beneficial to health [33,34]. Nowadays, probiotics have attracted much interest in the scientific community due to their therapeutic effects against many pathogens, their effectiveness in storage, their ability to persist in the gastrointestinal tract, and their non-toxicity. For these benefits, it is preferable to use probiotics instead of chemical additives and antibiotics [31,35]. In food technology, pasteurization, heating, drying, and salt addition are the most applied techniques to control microbial growth. A recent alternative process is based on the use of auxiliary LABs that have antimicrobial properties against harmful microorganisms to ensure food safety and human health [19,36].

L. Lactis possesses antioxidant activity due to the presence of various compounds like peptides, organic acids, and exopolysaccharides [37]. Studies have shown that *L. lactis* can produce superoxide dismutase as an antioxidant enzyme, which can scavenge free radicals and reduce oxidative stress [38,39]. In addition, *L. lactis* can produce other antioxidant compounds like phenolic

compounds, flavonoids, and carotenoids with potent antioxidant activity [40,41]. Furthermore, *L. lactis* has been found to improve the antioxidant capacity of dairy products during fermentation [42]. Indeed, *L. lactis* can produce metabolites with antioxidant activity during fermentation, thus increasing the bioavailability of antioxidants in the food matrix [43]. Overall, the antioxidant activity of *L. lactis* has important implications for human health and the food industry [44]. *L. lactis* can be used as a probiotic to improve gut health and can be built-in into food products to improve their nutritional value and shelf life [45].

This study aimed to assess the ability of *L. lactis* to inhibit the growth of *S. aureus* ATCC 29213, evaluate its antioxidant activity, characterize its sensitivity to antibiotics, and determine its suitability as a probiotic culture in food technology.

2. Material and methods

2.1. Strains and culture conditions

Twenty-three LAB strains were isolated from raw cow's milk and selected to be studied in the present work, namely *L. Lactis* subsp *lactis* (n = 10), *L. Lactis* ssp. *Cremoris* (n = 6), *L. Lactis* subsp *lactis biovardiacety lactis* (n = 7).

The strains L. *Lactis* were cultured on Man, Rogosaet Sharpe (MRS) Agar and identified using phenotypic and genotypic tests [46]. The isolates were then stored at −21 °C in an MRS broth supplement with 30 % (v/v) of glycerol, while multi-resistant bacteria were stored in Mueller-Hinton (MH).

Before starting, the search was revived several times. *L. lactis* strains were cultured in MRS broth at 30 °C for 24 h, and *S. aureus* ATCC *29213* at 37 °C for 24 h in MH agar. Three replicates were carried out for each replicate experience to confirm the results.

2.2. Culture medium for antibacterial tests

The antibacterial test was tested out in the Mueller Hinton agar (MHA) medium, widely used by several researchers [47,48]. In this research, the LAB cannot grow in the MHA medium. For this reason, we tried using adequate media for all tested strains, provided that they were inhibitor-free. The medium used in this test was MRS, which is suitable for both strains, *L. lactis* and *S. aureus* ATCC 29213.

2.3. Test of competitive nutritional interactions between L. lactis and S. aureus ATCC 29213

The antibacterial potential of *L. lactis* was screened by two different methods: competition for nutrients and disk diffusion techniques against *S. aureus* ATTC 29213, according to Suzuki and Suzuki [49], with some modifications. *S. aureus* ATCC 29213 was cultured in MHA and incubated for 24 h at 37 °C. After that, the cultures of *S. aureus* ATCC 29213 were adjusted with double distilled H_2O to a 0.5 McFarland standard using the nephelometer BD PhoenixSpec and then spread on MRS agar using a swab. The bacterial suspension of *L. lactis* was adjusted to different densities 0.5, 1, and 2 McFarland. The disks were filled with 40 μL of the supernatant and the bacterial suspension. The presence of antibacterial activity in the LAB culture supernatant was detected through the formation of a growth-inhibiting zone of the indicator strain around the disc (Fig. 4). The experiment was performed in triplicates and the results were reported in mean along with their standard deviation. Bacterial cell growth at each concentration (0.5, 1, and 2 Mcf) is determined by measuring the optical density (OD) at 540 nm using a spectrophotometer (vis-7220G).

2.4. Determination of the antibacterial components' nature

The antibacterial components' nature was determined according to the method described by Suzuki and Suzuki [49]. All strains with antibacterial activity were treated with NaOH, Catalase, and proteinase K. Culture broths of *L. Lactis* were centrifuged at 13000 rpm, 10 min, and 4 °C, and the cells were removed. Proteinase K stays active for a wide pH range from 7.5 to 12.0. The use of NaOH aims to neutralize bacterial supernatant, proteinase K to inhibit the bacteriocin effect, and the use of catalase to inhibit the hydrogen peroxide effect [50].

2.5. Testing of phenotypic antibiotic resistance

The susceptibility of *L. Lactis* strains were tested against twenty antibiotics such as Penicillin G (P1), Ampicillin (AMP 10), Amoxicillin (AML 25), Cefotaxime (CTX 5), Ceftriaxone (CRO 30), Cefepime (FEP 30), Imipeneme (IMP 10), Ertapeneme (ETP 10), Meropeneme (MEM 10), Teicoplanine (TEC 30), Vancomycine (VA 5), Vancomycine (VA 30), Clindamycine (DA 2), Sulfamethoxazole-trimethoprim (SXT 25), Gentamicine 30 (GN 30), Gentamicine 500 (GN 500), Streptomycin (S 300), Rifampicin (RD 30), Tetracycline (TE 30), and Chloramphenicol (C 30). According to the method of Vasiee et al. [51] with modifications, the turbidity of the bacterial suspensions of *L. Lactis* strains was adjusted to a density of 0.5 McFarland with the use of the same bacterial dose of all strains. Then, the strains were placed on the surface of MRS medium agar using the swabbing technique. The antibiotic discs were then placed on plates and incubated at 37 °C. The diameters of the areas around the disc were measured after the 24-h incubation period. Antibiotic discs are commercially available paper discs containing the appropriate dose of antibiotics [52].

2.6. Assessment of the antioxidant activity

The antioxidant activity of *L. lactis* was tested by 2,2-diphenyl-1 picrylhydrazyl (DPPH) according to the method of İncili et al. [53]

and Łepecka et al. [54] with some modifications. 60 μL of the cell-free supernatant (CFS) of the *L. lactis* obtained by cultures in MRS broth incubated for 6 days at 30 °C, centrifuged at 4000×*g* for 10 min, and added to 1.94 mL of freshly prepared DPPH (4 mg/L in ethanol). The compound was incubated for 30 min in darkness and measured using a spectrophotometer (vis- 7220G) at 517 nm. The experiment was performed in triplicate, and the results of DPPH inhibition were expressed as mean ± standard deviation and calculated using the following formula:

$(Abs_{DPPH}–Abs_{L.\ lactis}/Abs_{DPPH}) * 100.$

Abs_{DPPH}: absorbance of DPPH.

$Abs_{L.lactis}$: absorbance of the supernatant of bacteria.

2.7. Statistical analysis

All experiments were carried out in triplicate, and results were given as mean ± standard deviation.

Table 1
L. Lactis against *S. aureus* ATCC 29213.

		Inhibition diameters of mm			Bacterial suspension	Native supernatant	Supernatant treated by proteinase K	Supernatant treated by NaOH	Supernatant treated by Catalase
		0.5 McF	1 McF	2 McF					
L. lactis sp. *lactis*	Lc L5	18 ± 2	20 ± 1	20 ± 3	30 ± 3	29 ± 1	NI	26 ± 2	25 ± 3
	Lc L 6	17 ± 1	20 ± 2	20 ± 4	28 ± 3	26 ± 2	NI	23 ± 2	24 ± 1
	Lc L 9	16 ± 2	23 ± 3	27 ± 2	29 ± 2	26 ± 1	NI	24 ± 1	23 ± 2
	Lc L 10	19 ± 4	24 ± 1	24 ± 2	27 ± 4	24 ± 3	NI	24 ± 2	24 ± 1
	Lc L 11	19 ± 3	22 ± 2	25 ± 1	25 ± 2	23 ± 2	NI	21 ± 1	22 ± 1
	Lc L 15	23 ± 2	26 ± 3	30 ± 2	33 ± 4	31 ± 3	NI	28 ± 2	29 ± 1
	Lc L 18	14 ± 1	20 ± 1	25 ± 2	29 ± 3	27 ± 2	NI	25 ± 1	26 ± 1
	Lc L 19	28 ± 3	36 ± 2	36 ± 2	39 ± 3	36 ± 2	NI	34 ± 1	35 ± 1
	Lc L 21	28 ± 3	32 ± 1	35 ± 1	37 ± 2	36 ± 1	NI	35 ± 1	35 ± 1
	Lc L 26	31 ± 1	35 ± 3	40 ± 3	40 ± 3	39 ± 3	NI	34 ± 2	38 ± 2
L. lactis sp. *cremoris* 2	Lc C 1	11 ± 1	15 ± 1	18 ± 1	18 ± 2	16 ± 1	NI	15 ± 1	15 ± 1
	Lc C 2	13 ± 2	16 ± 3	19 ± 3	20 ± 2	19 ± 2	NI	18 ± 1	19 ± 1
	Lc C 3	12 ± 3	16 ± 2	18 ± 1	19 ± 3	17 ± 2	NI	16 ± 1	17 ± 1
	Lc C 4	14 ± 2	20 ± 1	20 ± 2	21 ± 3	20 ± 2	NI	18 ± 1	19 ± 2
	Lc C 8	12 ± 2	15 ± 2	15 ± 2	15 ± 2	14 ± 1	NI	13 ± 1	13 ± 2
	Lc C12	24 ± 1	25 ± 1	25 ± 2	30 ± 3	30 ± 2	NI	27 ± 1	27 ± 3
L. lactis sp. *lactisbiovar. diacetylactis*	Lc D 7	15 ± 3	24 ± 1	25 ± 2	30 ± 2	27 ± 3	7 ± 4	24 ± 1	25 ± 00
	Lc D 13	18 ± 2	20 ± 2	21 ± 2	18 ± 4	15 ± 3	9 ± 3	13 ± 1	14 ± 2
	Lc D 14	20 ± 2	28 ± 2	28 ± 3	30 ± 2	28 ± 2	7 ± 3	26 ± 1	26 ± 1
	Lc D 22	30 ± 2	35 ± 3	37 ± 3	39 ± 1	37 ± 1	8 ± 2	30 ± 2	32 ± 1
	Lc D 24	25 ± 2	27 ± 3	30 ± 3	30 ± 4	29 ± 3	8 ± 3	23 ± 2	26 ± 1
	Lc D 25	23 ± 3	25 ± 2	25 ± 3	25 ± 3	24 ± 2	7 ± 4	19 ± 1	21 ± 1
	Lc D 27	20 ± 3	25 ± 2	27 ± 2	27 ± 3	23 ± 2	9 ± 2	22 ± 2	22 ± 3

NI: No Inhibition.

3. Results

LAB genus *Lactococcus* presenting diversity in the species (*L. Lactis* subsp *lactis*, *L. lactis* ssp. *Cremoris,* and *L. Lactis subs plactis biovardiacety lactis*) were isolated and selected from raw cow's milk.

3.1. Optimization of culture medium for the antibacterial test

According to the result, it has been found that the use of the MRS medium was more efficient for the growth of *L. lactis* and *S. aureus* ATTC 29213 than MHA. Among the 23 screened strains, *L. Lactissubsp. lactis* showed higher antibacterial activities than *L. Lacti* ssp. *lactisbiovar. Diacetylactis* and L. *lactis sp. Cremoris* against *S. aureus* ATCC 29213. The antibacterial activity varied between strains for all tested subspecies. The results of *L. lactis* strains tested for their antibacterial activities against *S. aureus* ATCC 29213 are shown in Table 1.

The highest antibacterial activity was found in the stock solution of bacterial suspension of *L. lactis* sp. *Lactis* LC26 against *S. aureus* ATCC 29213 reached a value of 40 ± 3 mm, while the low antibacterial activity was reflected by a value of 25 ± 2 mm in the same subspecies. For the L. *lactis sp. Lactisbiovar diacetylactis* strains, the highest antibacterial activity against *S aureus* ATCC 29213 was 39 ± 1 mm of inhibition, and the lowest was 18 ± 4 mm. For the inhibition diameter recorded in the *L. lactis* sp. *Cremoris* strains, the maximum value was 30 ± 3 mm, while the minimum was 15 ± 2 mm.

Table 1 shows that, for most of the strains, the more the concentration of the bacterial suspension increases, the more the inhibition zone diameter increases, except for the Lc C8 strain in which the inhibition zone remained the same (15 mm) among the three used densities: 1, 2 McFarland, and stock solution of the bacterial suspension. We tried to determine a concentration of 0.5 Mcf for the two genres of bacteria *L. Lactis* and *S. aureus* ATCC 29213. Additionally, 1 and 2 Mcf are used to confirm that the increase in the concentration of *L. Lactis* induces the increase of the zone of inhibition. Fig. 1 shows that the more the concentration of strains *L. Lactis* increases, the more the bacterial rate increases.

The inhibition zones of native bacterial supernatants ranged between 23 ± 2 mm −39 ± 3 mm, 15 ± 3 mm −37±1 mm, and 14±1 mm −30±2 mm, respectively, for *L. Lactis* subsp. *Lactis, L. lactis* sp. *Lactisbiovar. diacetylactis, and L. lactic* ssp. *Cremoris* against *S. aureus* ATCC 25923.

Our results revealed the antibacterial activities of all strains of *L. lactis* sp. *lactis* and *L. lactis* sp. *Cremoris* since they were inactivated entirely by proteinase K, which is in agreement with the study realized by Karakas-Sen and Karakas [55]. For L. *lactis sp. lactisbiovar. diacetylactis*, they are partially inactivated by proteinase K. The inhibition zone of all *L. Lactis* strains was reduced after treating the supernatant with catalase, and neutralization inhibition remained but at a reduced diameter. This result is similar to the research realized by Ref. [19].

3.2. Antibiotic resistance

The results of *L. lactis* strains tested for antibiotic resistance are shown in Fig. 2. The results indicate that all strains of *L. lactis* were resistant to Cefotaxime, Sulfamethoxazole, trimethoprim, and Streptomycin, while they were sensitive to Ampicillin, Amoxicillin, Penicillin G, Teicoplanin, Vancomycin, Gentamicin 500, Tetracycline, and Chloramphenicol. The strains of *L. Lactissubsp. Lactis* were resistant to Meropeneme (10 %), Ceftriaxone (10 %), Imipenem (10 %), Cefepime (60 %), Erthapenem (90 %), and clindamycin (100 %).

The L. *lactis sp. Cremoris* were resistant to Rifampicin (16.6 %), Imipeneme (33.3 %), Cefepime (66.6 %), Ertapeneme (83.3 %), and Clindamycine (83.3 %). The L. *lactissp.diacetylactis*strains were resistant to clindamycin (14 %), Ceftriaxone (28.5 %), Meropeneme (42.8 %), Imipeneme (42.8 %), Cefepime (85.7 %), and Ertapeneme (100 %).

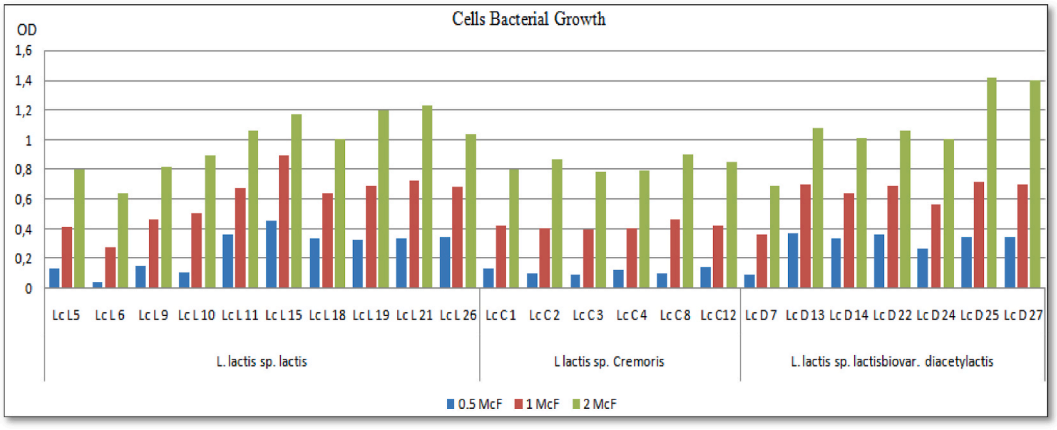

Fig. 1. Optical density of each bacterial concentration of *L. lactis* at 540 nm.

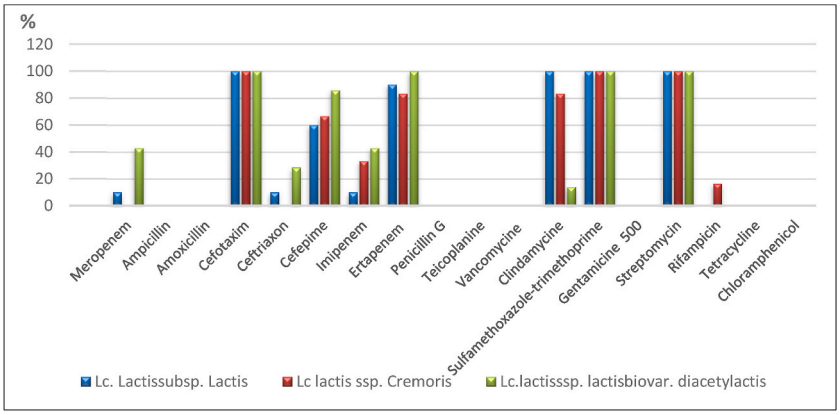

Fig. 2. Resistance percentage of *L. lactis* to antibiotics.

3.3. Antioxidant activity

The DPPH inhibition activities varied between strains of *L. lactis* ranged from 10.56 ± 1.28 % to 26.29 ± 0.05 % after 1 week of fermentation (Fig. 3). In this study, the antioxidant activity of *L. lactis sp. Lactisbiovar diacetylactis* was between 18.10 ± 0.15 % and 26.29 ± 0.05 %, higher than *L. lactis* sp. *lactis* 16,61 ± 0.76 % −24,63 ± 0.24 %, followed by *L. lactis sp. Cremoris* (10.56 ± 1.28 % −22.29 ± 0.27 %).

4. Discussion

The use of LAB as an inhibitor against undesirable microorganisms is widely practiced [27,56], given their experimentally proven antibacterial efficacy [57]. Two methods were proposed in the study of the inhibition of *S. aureus* ATCC 29213 by L. *Lactis*: competition for nutrients and components in nature. A nutritional competition test was employed to determine whether *L. Lactis* antagonizes *S. aureus* ATCC 29213. Nutrition is vital to the survival of any organism [55]. In a state of stress caused by nutritional limitation, LAB produces diverse antimicrobial substances like hydrogen peroxide, lactic acid, diacetyl, carbon dioxide, acetic acid, and bacteriocins [14]. Since they inhibit other microorganisms, these produced molecules ensure the survival of LAB and allow better absorption of nutrients, thus involving a mechanism known as quorum sensing [47]. Quorum sensing is important for bacterial stress response [47]. When environmental information such as pH, nutrient availability, temperature, and cell population density is transduced into the bacterial cell, the molecule synthesis signal is stimulated. When the concentration of small molecules reaches a threshold, the corresponding target genes are activated, and drugs are produced [58].

In this research, we observed that the MRS medium is adequate for testing competitive nutritional interactions between *L. lactis* and *S. aureus* ATCC 29213. *L. lactis* inhibited *S. aureus* ATCC 29213 from absorbing nutrients, and the inhibition zones ranged between 15 mm and 40 mm. LAB require specific nutrients for their growth and development. The two-culture media mentioned, MRS and MH, have different compositions and provide different elements for the growth of LAB. MRS medium is a rich culture medium that contains

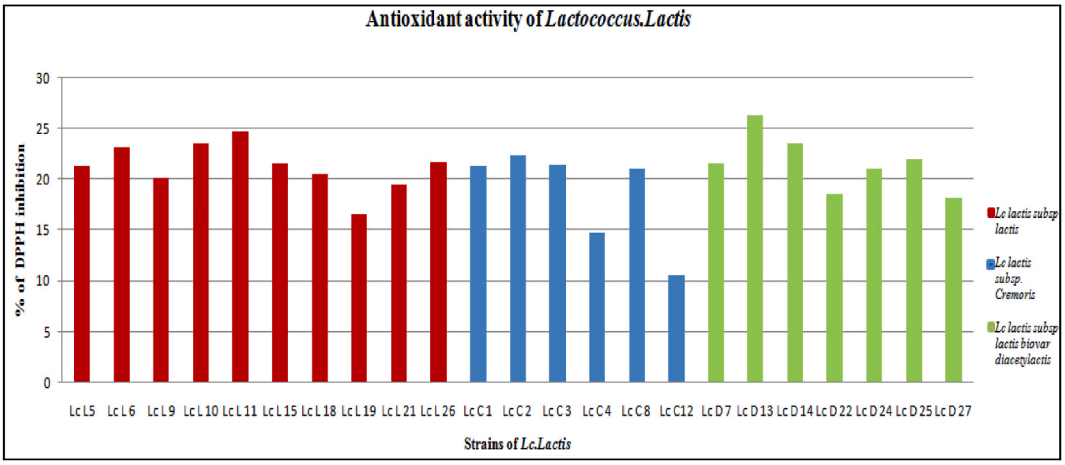

Fig. 3. Antioxidant activity of *L. lactis* strains.

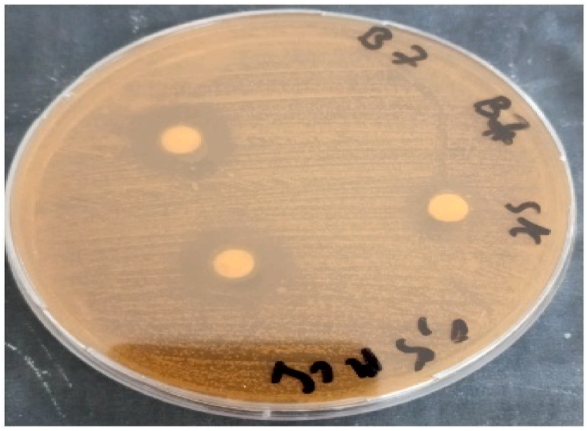

Fig. 4. Antibacterial activity of the *L. lactis* sp. *lactisbiovar. diacetylactis* against *S. aureus* ATCC 29213.

peptone, yeast extract, meat extract, glucose, tween 80, sodium acetate, magnesium sulphate, manganese sulphate, and disodium phosphate. These components provide a wide range of nutrients, such as amino acids, vitamins, minerals, and energy sources (glucose) that support the growth and development of LAB. On the other hand, MH medium is a less rich culture medium that includes acid hydrolyzate of casein (peptone), meat extract, starch, calcium, and magnesium. While it does provide some essential nutrients, it may not be sufficient for the optimal growth of LAB. The absence of specific components like yeast extract, glucose, sodium acetate, and manganese sulphate in the MH medium may limit the growth and development of LAB [59]. According to Tong et al. [47], *L. lactis* remains dominant in bacterial competition due to nisinbacteriocin. This cationic peptide can bind to many anionic lipids in the plasma membrane of gram-positive bacteria [60].

In situations where different bacterial species vie for attachment to epithelial cells through receptors, probiotics are advantageous for human health by impeding the adhesion and proliferation of harmful bacteria. Probiotics engage in competition for vital nutrients necessary for their growth and reproduction, which would otherwise be utilized by pathogens. For instance, probiotics can surpass pathogens in the consumption of monosaccharides, hindering the growth of organisms. This competitive edge slows down the proliferation of pathogenic microbes, leading to a decrease in their population within the gastrointestinal tract [61].

Our results revealed that all *L. lactis* strains showed antibacterial activity against *S. aureus* ATCC 29213 (Fig. 4), with different proportions 0.5, 1, 2 McFarland, and stock solution of bacterial suspension, which agrees with the result of Alomar et al. [62]. Also, all strains of *L. lactis* competitively inhibited the growth of *S. aureus* ATCC 29213 under the presence of nutrients. It is observed that the antibacterial activity in the bacterial suspension of all strains of *L. Lactis* was higher than the supernatant activity of the same strain. That signified this activity relates to the competition for nutrients with harmful bacteria. Nutrients, space, and antimicrobial active metabolites are considered among the antimicrobial properties of LAB [14,32].

L. lactis produces a multitude of antibacterial compounds like hydrogen peroxide, lactic acid, and bacteriocins [29], responsible for their antimicrobial effect as demonstrated in several studies [63]. *L. lactis* can produce an antimicrobial agent known as lactic acid. This latter is an organic acid that acts as a bacteriostatic agent, particularly on pathogenic bacteria [64]. Inhibition effects due to lactic acid, hydrogen peroxide (H_2O_2), and protein were ruled out by adding NaOH, Catalase, and proteinase K [65,66].

The goal of using NaOH is to eliminate acid production that could inhibit pathogenic bacteria in the supernatant [67]. In our research, all tested strains of *L. lactis* slightly decreased their antibacterial activity after acid elimination. Charlier et al. [68] researched the effect of two groups of *L. lactis* strains that showed strong (pH 4.5) and low acidification (pH 5.8) against *S. aureus*. They found that both groups exerted strong inhibition against *S. aureus,* except 7 % of strains with weak acidification showed weak inhibition. These findings explain that there is an intra-species variability of *L. lactis* strains when screening for their inhibition capacities against the growth of *S. aureus,* and that most *Lactococcus* strains have high antagonistic effects [68]. The research by Ren et al. showed that acid production is the main antimicrobial effect in certain strains and confirmed that the bacteriostatic effect is due to hydrogen peroxide [67]. When catalase is used, the zone of inhibition decreases, which explains why our strains produce hydrogen Peroxide. In the same context, Reis et al. showed that hydrogen Peroxide produced by LAB can inhibit the growth of pathogenic microorganisms and is considered bactericidal depending on the concentration used and environmental factors [27].

In this research, the antibacterial activities of the *L. lactis* sp. *lactis*, and *L. lactis* sp. *cremoris* treated with proteinase K were completely inactivated. A comparable result was previously reported by Karakas and Karakas [55]. That signified the proteinaceous nature of the antibacterial substances produced by these strains established their sensitivity to proteolytic enzyme protease. Silva et al. [15]reported that *Lactococcus* strains inhibit pathogenic strains by producing bacteriocins referred to as lacticins, which are specifically produced by *L. lactis.* According to Benítez-Chao et al. [69], bacteriocins are a proteinaceous and promising group of antimicrobial peptides produced by bacteria. For L. *lactis sp. Lactisbiovar diacetylactis,* the inhibition zone is reduced after using proteinase K. This means antibacterial substances may contain other compounds with antibacterial activity. According to Fusieger et al. [13], *L. lactis* subsp. *lactisbv. diacetylactis* can produce an antimicrobial compound, which is diacetyl [70].

The screening for virulence factors encoding genetic and phenotypic determinants of LAB resistance to different antibiotics was

evaluated and tested to guarantee LAB safety for food applications [33,71]. Probiotic strains should be carefully selected and monitored to ensure that they do not pose a risk of acquired antibiotic resistance [33]. The obtained antibiotic resistance results showed that all strains were sensitive to Amoxicillin, Ampicillin, Chloramphenicol, Gentamicin, Penicillin G, Teicoplanin, Tetracycline, and Vancomycin. These results are consistent with Ramalho et al. [72], who showed the presence of intrinsic genes of resistance to antibiotics in our strains, indicating the absence of acquired resistance. Acquired antibiotic resistance can be transmitted to other bacteria by horizontal gene transfer since it is encoded by genes located on mobile genetic elements (plasmids or transposons) [52]. In contrast, antibiotic resistance cannot be transmitted from bacteria to other bacteria because it is an inherent, intrinsic trait encoded by genes located on chromosomal DNA [52].

FAO/WHO treats the problem of antibiotic resistance genes in probiotic strains, which shows that one of the most important parameters for selecting an LAB probiotic is the capacity to transfer antibiotic resistance genes. Still, their use should not be allowed [73].

L. lactis has been found to possess antioxidant activity, which may contribute to its health-promoting properties [72]. The strains of *L. lactis* were tested for their antioxidant activity after 1 week of fermentation. The antioxidant activity of *L. lactis* towards the DPPH radical was evaluated with a spectrophotometer after reducing this radical, measuring the violet (DPPH•) to yellow (DPPH-H) transition at 517 nm. The result demonstrated the DPPH scavenging activity with an inhibition rate in the range of 10.56 ± 1.28 % and 26.29 ± 0.05 %. Similar results have been reported by Uugantsetseg and Batjargal [74], with a value of 15.87 % for DPPH inhibition activity in *L. lactis*. The antioxidant activity of *L. lactissp.lactisbiovar. Diacetylactis* (18.10 ± 0.15–26,29 ± 0.05) was higher than that of *L. Lactis* sp. *Lactis* (16.61 ± 0.76 % - 24.63 ± 0.24 %), followed by *L. lactic* ssp. *cremoris* that ranged between 10.56 ± 1.28 % and 22.29 ± 0.27 %.

Uugantsetseg and Batjargale [74] explained that antioxidant activity increased with the fermentation time. *L. lactis* has been shown to produce a panoply of antioxidant compounds, including carotenoids, flavonoids, phenolic acids, exopolysaccharides, peptides, and organic acids [72,75]. For example, a recent study found that yogurt fermented with this bacterium had higher antioxidant activity than yogurt fermented with other strains that do not have these properties [76].

Exopolysaccharides are complex carbohydrates produced by bacteria that have been found to have antioxidant activity [77,78]. They can scavenge free radicals and prevent cell oxidative damage [79]. Peptides are short chains of amino acids with antioxidant properties [80]. They can protect against oxidative stress and can inhibit the production of reactive oxygen species [81]. Organic acids, like lactic acid, are produced by *L. lactis* through fermentation and can also act as antioxidants by reducing oxidative damage to cells [82].

5. Conclusion

This study revealed that *L. lactis* strains display antibacterial activity through the production of various compounds, including hydrogen peroxide, lactic acid, diacetyl, and bacteriocins. These compounds effectively inhibited the growth of multi-resistant bacteria, particularly *S. aureus* ATCC 29213. Among the tested strains, Lc 26 exhibited significant antibacterial activity potential. Additionally, *L. lactis* strains demonstrated antioxidant activity, with DPPH radical scavenging activity ranging from 10.56 ± 1.28 % to 26.29 ± 0.05 %. Notably, LC 13 showed high antioxidant potential, indicating its possible use in alleviating oxidative stress. It is noteworthy that *L. lactis* strains exhibited natural resistance to antibiotics, which could be advantageous for their potential use as probiotics or starters. Future research will focus on extracting bacteriocins from *L. lactis* to evaluate their effectiveness against microorganisms such as *Listeria*, *Citrobacter*, *E. coli*, Yersinia, and Klebsiella.

Ethics approval and consent to participate

Not applicable.

Data availability statement

The data used to support the findings of this study were included in the article.

CRediT authorship contribution statement

Nora Hamdaoui: Writing – review & editing, Writing – original draft, Conceptualization. **Chaymae Benkirane:** Writing – original draft, Formal analysis, Data curation. **Haytham Bouaamali:** Writing – original draft, Methodology, Investigation. **Ali Azghar:** Writing – original draft, Software, Resources. **Mohamed Mouncif:** Writing – original draft, Visualization, Validation. **Adil Maleb:** Writing – review & editing, Writing – original draft, Visualization. **Belkheir Hammouti:** Writing – review & editing, Writing – original draft, Formal analysis. **Khalid Mashay Al-Anazi:** Writing – review & editing, Resources. **Pankaj Kumar:** Writing – review & editing, Writing – original draft, Software, Methodology, Data curation. **Krishna Kumar Yadav:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Jeong Ryeol Choi:** Writing – review & editing, Resources, Funding acquisition. **Mustapha Meziane:** Writing – review & editing, Resources, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of Abbreviations

Abs Absorbance
ATCC American Type Culture Collection
CFS Cell-free supernatant
DPPH 2,2-diphenyl-1-picryl-hydrazyl
GRAS Generally recognized as safe
LAB Lactic acid bacteria
MH Muller Hinton
MRS Man, Rogosaet Sharpe
S. aureus *Staphylococcus aureus*
US American

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