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Biocontrol of *Bacillus cereus* by *Lactobacillus plantarum* in Kareish cheese and yogurt

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ABSTRACT

This study aims to biocontrol of *Bacillus cereus* by *Lactobacillus plantarum* in Kareish cheese and yogurts. The minimum inhibitory concentrations (MICs), antioxidant potentials, total flavonoids content (TFC) and total phenolic content (TPC) of *L. plantarum* were also estimated. Results showed that incidence of *B. cereus* in Kareish cheese and yogurt was 16 and 4%, respectively. Four virulence genes were investigated by PCR in *B. cereus* isolates (n = 10). Two toxin producing genes, cytotoxin K (*cytK*), and phosphatidylcholine-hydrolyzing phospholipase C (*Pc-plc*), were detected in all *B. cereus*, whereas enterotoxigenic (*nhe*) and hemolysin BL (*hbl*) genes were detected in 90 and 50%, respectively. All isolates were vulnerable to erythromycin and gentamicin (100%) with intermediate sensitivity to ciprofloxacin and complete resistance to tetracycline (100%). *L. plantarum* showed antibacterial power against *B. cereus* EMCC1006 reference strain with MIC at 3.1 mg/mL. From the different concentrations (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL) of *L. plantarum* which mixed with Kareish cheese and yogurt samples, the minimum concentrations displayed the excellent sensory parameters. TPC and TFC of *L. plantarum* CFS were 18.5 (μg GAE/g) and 2.67 (μg QE/g), respectively. Regarding antioxidant activity, IC_{50} of *L. plantarum* was 53.84 μg/mL, while IC_{50} of ascorbic acid was 26.36 μg/mL. In sum, *L. plantarum* could be used as a promising antibacterial and antioxidant agent for biocontrol of *B. cereus* to ensure dairy safe without negative impact on sensorial attributes.

1. Introduction

Bacillus cereus is one of the popular foodborne pathogens in foods (Rahnama et al., 2023). *Bacillus cereus* is an endospore-forming aerobic organism that is widely spread in the environment with significant importance in the dairy products (Jovanovic, Ornelis, Madder, & Rajkovic, 2021; Zhou et al., 2023). *B. cereus* pathogenicity depends on the production of numerous exogenic enzymes like hemolysins, phospholipases, proteases, and the ability to form biofilms, as well the presence of toxin-encoding genes (Dietrich, Jessberger, Ehling-Schulz, Märtlbauer, & Granum, 2021; Torii & Ohkubo, 2023). Occurrence of *B. cereus* in food and dairy is responsible for food-poisoning disease associated with diarrhea, emesis, or food-allied gastrointestinal diseases (Li et al., 2023). Diarrhea is allied with production of heat-labile toxin in the small intestine for example cytotoxin K (*cytK*), enterotoxin FM, the enterotoxin (*nhe*) without haemolysis, and hemolysin BL (*hbl*). On the other hands, the emetic syndrome is mainly caused by a lethal-heat resistant toxins termed "cereulide", which is synthesized by a non-ribosomal peptide synthetase (NRPS) encoded by a *ces* gene (Dietrich et al., 2021; Tuipulotu, Mathur, Ngo, & Man, 2021).). The phosphatidylcholine-specific phospholipase C and sphingomyelinase encoded by the *plc* and *sph* genes, respectively composed related to the hemolytic activity in

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B. cereus isolates (Tirloni et al., 2023). In addition, the fourth most common reason of the foodborne epidemic in the European Union (EU) has been *B. cereus*; according to European Centre for Disease Prevention and Control (ECDC), and European Food Safety Authority (EFSA) (Rahnama et al., 2023).

Yogurt and Kareish cheese have a wide popularity for consumers for their nutritional and health benefits. In Egypt, Kareish cheese is considered as the most popular soft cheese due to its low fat, high protein content in addition to its reasonable price in relation to other cheeses (Hassan, Korany, Zeinhom, Mohamed, & Abdel-Atty, 2022). *B. cereus* is likely to be ubiquitous in the dairy farm environment. Contamination of dairy products with *B. cereus* has a great effect on both consumer safety and shelf-life of dairy products (Elafify et al., 2023). The plentiful occurrence of *B. cereus* in normal environment and its capability to form endospore allows this bacterium to survive in severe environmental circumstances that lead to endurance in a wide range of pH and temperatures. Hence, the capability of *B. cereus* cells to attach to the surfaces of stainless steel used in food products handling and processing (Rahnama et al., 2023). The ability of *B. cereus* to form biofilm and spores makes it highly resistant to food preservatives and antimicrobial candidates, posing persistent hazards to food industry and the humans health (Li et al., 2023). Furthermore, *B. cereus* spores are able to live in pasteurization treatment and cooking processes due to of their high thermal stability (Rodrigo, Rosell, & Martinez, 2021).

Many Lactic Acid Bacteria (LAB) are considered probiotics and present numerous health benefits via modulating the gut microbiota and metabolites (Fang et al., 2023). Hence, LAB used as food preservative in food products (Hamad, Omar, et al., 2022). *Lactobacillus plantarum* was reported to have antagonistic effect against pathogenic and spoilage organisms in food (Siedler, Balti, & Neves, 2019; Tian et al., 2022). Recently, functional food products i.e., probiotic fermented foods, are receiving extra consideration owing to the consciousness of consumers to the nutritious food product for the elevation of good health against several diseases (Ali et al., 2023; Hussain et al., 2023). To date, there are very few investigations offered concerning the incidence and controlling of foodborne pathogens *Bacillus cereus* in Kareish cheese and yogurt. Therefore, this study aims to investigate the incidence of *B. cereus* in Kareish cheese and yogurt combined with virulence genes detection in the isolated strains. Additionally, antibacterial susceptibility profile of *B. cereus* isolates for some antimicrobial agents was detected. Finally, antibacterial activity and antioxidant potentials of cell free supernatant of *L. plantarum* EMCC 1027 were evaluated as an approach for controlling *B. cereus* in Kareish cheese and yogurt products.

2. Materials and methods

2.1. Strains and collection of samples

A reference pathogenic *B. cereus* EMCC1006 strain and *L. plantarum* EMCC 1027 were gotten from (Cairo Microbiological Research Center, Cairo MIRCEN), College of Agriculture, University of Ain Shams, Cairo, Egypt. One hundred samples of Kareish cheese and yogurt (50 each) were randomly purchased from different retail markets at Alexandria governorate, Egypt. All samples were collected in sterile containers and transferred instantly in ice-box for further isolation and investigation of *B. cereus.*

2.2. Isolation and identification of B. cereus

For quantitative detection purpose of *B. cereus*, the approach of Amor et al. (2018) was followed. Firstly, 10 g from each sample were homogenized (Homogenizer, ThermoFisher Scientific Co., Egypt) in 90 mL sodium citrate at 2500×*g*/2 min under an aseptic conditions. After, the homogenate sample was mixed with 90 mL of 0.1% buffered peptone water (BPW) and incubated at 35 °C/24 h. A loopful of the incubated mix was streaked on Mannitol Yolk Polymyxin agar (MYP) (bought form Oxoid, UK) and incubated at 37 °C/24 h. The suspected *B. cereus* colonies with large size diameter (3–7 mm), pink colored and surrounded by a good zone of egg yolk precipitation of the same color, were purified, subcultured on BHI-YE agar (gotten from Fisher Bioblock, France) and incubated at 30 °C/24 h. Each colony was transferred into Tryptic Soy Broth (TSB) broth (AES Laboratory, France) and incubated at 30 °C/24 h. Finally, the cultures at a final concentration of 25% were frozen at −80 °C after adding of glycerol (purchased from Sigma Aldrich, Saint Quentin Fallavier, France).

2.3. Molecular identification of B. cereus isolates and detection of its virulence genes

2.3.1. DNA extraction

Using QIAamp DNA Mini kit (obtained from Qiagen, Hilden, Germany); DNA extraction from the purified colonies was carried out according to the procedure of Hamad, Hafez, et al. (2023) with slight amendments. Firstly, 200 μL of each colony suspension was incubated at 56 °C/10 min with (10 μL of proteinase K) and 200 μL of lysis buffer. A 200 μL of ethel alcohol was incorporated to the lysate after the incubation. Afterword, the mix washed and centrifuged. Nucleic acid was eluted with (100 μL of elution buffer) provided in the kit.

2.3.2. PCR amplification

The primers used in PCR reaction in this study were supplied from Metabion (Planegg/Steinkirchen, Germany) according to Ehling-Schulz et al. (2006); Martínez-Blanch, Sánchez, Garay, and Aznar (2009) in (Table S1). PCR reaction mixture (25 μL) included (12.5 μL) of EmeraldAmp Max PCR Master Mix (Model Takara, Japan), 1 μL of each forward and reverse primer (20 pmol), (5.5 μL) of water, and (5 μL) of DNA template. The PCR reaction was conducted in an Applied biosystem 2720 thermal cycler. The amplicon was separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). Gene ruler 100 bp ladder (Fermentas, Germany) and gelpilot100 bp plus ladder (Qiagen, Gmbh, Germany) were utilized to determine the fragment sizes. Further, the gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

2.4. Antimicrobial activity

Agar well diffusion approach was utilized to assess the antimicrobial activity of four antimicrobial agents i.e., (Ciprofloxacin, Erythromycin, Gentamicin and Tertracyclin) against 10 *B. cereus* isolates according to Serrano Cardona and Muñoz Mata (2013). Muller-Hinton agar plate surface was inoculated with the isolated strains. A total volume (20–100 μL) of two-fold serial dilutions of each antimicrobial agent at desired concentration (0.5, 1, 2, 4 μg/mL) was introduced into each well. Then, agar plates were incubated at 35 ± 2 °C (New Brunswick™ Galaxy® 170 R CO2 Incubator Series, Eppendorf, Spain) for 16–20 h. Finally, the minimum inhibitory concentrations (MICs) were recorded.

2.5. Antibacterial activity of L. plantarum EMCC 1027 against B. cereus

2.5.1. Preparation of L. plantarum EMCC 1027 CFS

A colony suspension of *L. plantarum* EMCC 1027 in phosphate buffer saline (PBS; obtained from Sigma Aldrich, Germany) was adjusted to 0.220 (OD600). Then, 1 mL of each suspension was inoculated into 9 mL of deMan Rogosa Sharpe (MRS) broth and incubated at 37 °C/24 h. Each probiotic culture was enriched in 200 mL MRS broth, incubated with shaking under aerobic conditions at 37 °C/48 h. Further, the culture was centrifuged at 10.000×*g* for10 min and the resulted supernatant was collected and filtered by a 0.2 mm syringe filter membrane, then, the culture was transferred into a conical tube under aseptic conditions and stored at −80 °C. The supernatant was then lyophilized (model FDF 0350, Republic of Korea). Several concentrations of *L. plantarum* i.e., (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL) were prepared by serial

dilution in Milli-Q water (Siemems, Ultra Clear UV UF TM, Germany) (Hamad, Ombarak, et al., 2022).

2.5.2. Antibacterial activity of L. plantarum EMCC 1027 CFS against B. cereus

L. plantarum capability to inhibit the growth of both 10 *B. cereus* isolates and 2 *B. cereus* EMCC1006 as a reference strain was assessed via agar disk diffusion assay. Overnight cultures of *B. cereus* and the reference bacterium were diluted to 10^6 in order to obtain semi-confluent growth and inoculated over nutrient agar plates by sterile cotton swabs. After the drying process, *L. plantarum* CFSs were loaded to each separate disc (20 μL) and the plates were reserved at 4 °C for 30 min, then incubated at 37 °C/24 h. Finally, the formed clear zone was noted in mm is considered an antibacterial power of *L. plantarum* CFS (Hamad, Amer, et al., 2023).

2.5.3. The minimum inhibitory concentrations (MICs) of L. plantarum CFS against B. cereus reference strain

MICs of *L. plantarum* EMCC 1027 CFS against the reference *B. cereus* EMCC1006 strain were carried out by the descending ratios of *L. plantarum* EMCC 1027 CFS i.e., (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL). The reference strain suspension of growing cells was prepared in sterile saline solution and adapted to a density of (10^6 cells/mL). A plate of nutrient agar was inoculated with the reference strain suspension. The plates were then dried at room temperature (RT) for 15 min before applying the disks. Then, 10 mL of each concentration from *L. planetarium* CFS was separately used to impregnate the disks. The plates were incubated at 37 °C/24 h, and finally the MIC values were recorded in triplicates (Hamad, Ombarak, et al., 2022).

2.6. Determination of total phenolic content (TPC) of L. plantarum EMCC 1027 CFS

TPC of *L. plantarum* CFS, was analyzed via Folin-Ciocalteu assay (Hamad, Taha, El-Deeb, & Alshehri, 2015). Briefly, 0.1 mL Folin-Ciocalteu (Sigma-Aldrich, USA) was added to 0.1 mL reconstituted *L. plantarum* EMCC 1027 CFS. The mixture was allowed for 15 min, and then 2 mL of Na_2CO_3 (2 g/dL) was incorporated. Then, the blend was left at RT for 30 min. At 760 nm, TPC was measured using spectrophotometer (model Labo America, USA) using gallic acid as a standard. TPC was expressed as ((μg GAE)/g sample).

2.7. Determination of total flavonoids content (TFC) of L. plantarum EMCC 1027 CFS

For TFC of *L. plantarum* CFS measuring; the method of El Sohaimy, El-Sheikh, Refaay, and Zaytoun (2016) was followed. Briefly, 1 mL of the probiotic CFS and 4 mL of water (Milli-Q, Siemens Ultra Clear, Germany) were placed in a volumetric flask. Then, 0.75 mL of sodium nitrite (5 g/dL), 0.150 mL of $AlCl_3$ (10 g/dL) was added to the flask. After approximately 5 min incubation at RT, 0.5 mL of 1 mol/L of NaOH was added to the mixture. The absorbance was read at 510 nm by UV/VIS spectrophotometer (PG Instrument Ltd., UK). The TFC findings were expressed as milligram catechol equivalent per gram sample (μg QE/g).

2.8. Determination of antioxidant activity of L. plantarum EMCC 1027 CFS

The capacity of *L. plantarum* CFS to scavenge 2,2-diphenylpicrylhydrazyl (DPPH) free radicals was conducted according to the approach of El Sohaimy, Mohamed, Shehata, Mehany, & Zaitoun (2018). Each CFS solution was resolved in MetOH to 1 mg/mL. CFS serial dilutions were made as follow; 1 mL of each dilution was then mixed with (1 mL MetOH) solution of DPPH· in a (1 mg/mL) ratio. After that, the incubation for the mixture in darkness was done for 30 min. Furthermore, the absorbance was read at 517 nm spectrophotometrically. Ascorbic acid (AA) was used as a standard antioxidant. The findings were expressed as IC_{50} (defined as the CFS concentration which can inhibit the 50% DPPH free radical). The percentage of inhibition was calculated using the following equation:

$$Inhibition\ \% = \frac{\text{A of control} - \text{A of sample}}{A\ of\ control} \times 100 \quad (1)$$

where A: absorbance.

2.9. Identification of phenolic compounds in L. plantarum EMCC 1027 CFS by HPLC

HPLC (model Agilent 1260 infinity HPLC Series, Agilent, USA) was utilized for identification of phenolic substances in *L. plantarum* CFS according to Hamad, Ombarak, et al. (2022) following Agilent application note 2016 (publication number: 5991–3801 EN). HPLC was attached with Quaternary pump, a Zorbax Eclipse plus C18 column 100 mm × 4.6 mm i.d., (Agilent technologies, USA), and adjusted at 25 °C. Then, the separation was accomplished by a ternary linear elution gradient as follow; (1): H_2O- 0.2% H3PO4, (2): methanol, and (3): acetonitrile. All of these three reagents were HPLC grade. The injected volume was 20 μL, and VWD detector set at 284 nm was employed in the analyses.

2.10. Sensory evaluation of kareish cheese and yogurt prepared with L. plantarum EMCC 1027 CFS

L. plantarum EMCC 1027 CFSs at different concentrations (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL) were mixed with Kareish cheese and yogurt samples during the preparation stage. Sensory evaluation was carried out on the mixture by semi-trained panelists consisting of 25 persons. The panelists were demanded their references for color, odor, texture and overall acceptability attributes. The organoleptic assessment of the samples was performed at RT. The score for each item form the sensorial properties was conducted by 9-points descriptive scale (Hamad, Saad, et al., 2022).

2.11. Statistical analysis

All of the data were presented as mean ± standard deviations (SD) of triplicates. One-way ANOVA was performed with IBM SPSS Statistics software (version 23, USA). The statistical significance level was estimated at ($P < 0.05$).

3. Results and discussion

3.1. Occurrence of B. cereus in kareish cheese and yogurt samples

Contamination of dairy products with microbial pathogens is the most serious issue related to their safety and human's health all over the world. Furthermore, dairy products are highly sensitive to spoilage due to the milk and its products enhance the food microbe growth. *B. cereus* is one of the most crucial causes of food poisoning all cross the world due to of its enterotoxins (Han et al., 2023). Therefore, the incidence of *B. cereus* microbe and/or their toxins in food and dairy products is a serious hazard for the human health.

The current study revealed that the prevalence of *B. cereus* in Kareish cheese and yogurt samples was 16 and 4%, respectively (Table S2). Osama, Ahmed, Abdulmawjood, and Al-Ashmawy (2020) isolated *B. cereus* from 40% of the examined Kareish cheese samples while *B. cereus* was not recovered in any cheese sample tested by Heikal and Al-wakeel (2014) and Ibrahim, Sharaf, and El-khalek (2015). Moreover, previous study by Adam, Salwa, Aly, Saad (2021) reported the incidence of *B. cereus* in yogurt samples and recorded a ratio of 8%. Hence, 40% of positive *B. cereus* have been observed in Kareish cheese samples (Hefny,

Mohamed, Etokhy, & Abd El-Azeem, 2020).

Moreover, 4.0% of yogurt samples have contained on *B. cereus* from total 200 yoghurt samples (Fetouh, Ibrahim, El Barbary, & Maarouf, 2022). On the other hands, Tirloni, Ghelardi, Celandroni, Bernardi, and Stella (2017) failed to detect *B. cereus* in yogurt samples. Therefore, the varied detections rate of *B. cereus* may be due to the different microbial load and the low sanitation criteria during food chain and cheese preparation.

B. cereus is repeatedly isolated from the raw milk, and due to of its capability to resist pasteurization process, it is of special risk in the dairy industries, as the occurrence of diarrheal syndrome in dairy foods (Tirloni et al., 2017). The present study confirmed that, some of the examined Kareish cheese and yogurt samples have serious role in transmission of *B. cereus* to humans that considered as serious health risk. Indeed, the low sanitation level and low temperature processing of the curd of Kareish cheese is the main cause of contamination.

Overall, dairy products are extremely susceptible to *B. cereus* contamination due to the natural presence of the microorganisms in the environment, therefore, enabling the contamination of raw materials, and post-production or post-pasteurization contamination. This knowledge reports that strict cleaning management must be carried out to control *B. cereus* in order to assure high safety and quality dairy foods.

3.2. Prevalence of virulence genes of B. cereus isolates

In the present research, occurrence of some virulence genes in *B. cereus* isolates (Table 1, Fig. 1A, B, C, and D) from Kareish cheese revealed that *cytK* and *Pc-plc* genes were detected in all (100%) tested *B. cereus* isolates, followed by *nhe* gene (87.5%) then *hbl* gene which detected in only in (50%) of the tested isolates. Prevalence of the same virulence genes in *B. cereus* isolates from yogurt (Table 1, Fig. 1A, B, C, and D) displayed that *nhe, cytK,* and *Pc-plc* genes detected in all tested isolates while *hbl* gene recorded in 50% of the examined isolates. Eltokhy, Abdelsamei, El barbary, and Nassif, (2021) pointed that all *B. cereus* isolated from dairy products were positive for *nhe* and *cytK* genes. Further, Fetouh et al. (2022) reported that all of the detected genes were Enterotoxigenic strains that involved in food poisoning, as the two virulence genes cytotoxic K (*cytK*) and non-hemolytic enterotoxin (*nhe*) were amplified in all the examined isolates. In addition, *hbl*, *nhe*, and *cytk* genes were observed in all *B. cereus* isolates tested and recorded of 47%, 52%, and 33% respectively, however, *ces* gene was absent in all *B. cereus* isolates (Hefny et al., 2020).

3.3. Antibacterial activity of L. plantarum CFS against B. cereus isolates

The misuse of antimicrobial agents in dairy farms caused an increased bacterial resistance. As shown in Table 2, all *B. cereus* isolates were investigated for their susceptibility for four commonly used antimicrobial agents. All tested isolates showed 100% sensitivity to erythromycin, gentamicin, intermediate sensitivity to ciprofloxacin and 100% resistance to tetracycline.

Table 1
Prevalence of virulence genes of *B. cereus* isolates.

B. cereus isolate No.	*B. cereus* isolates source	Virulence genes			
		hbl	nhe	cytK	Pc-plc
1	Kareish cheese	+	+	+	+
2		+	+	+	+
3		+	+	+	+
4		−	−	+	+
5		−	+	+	+
6		−	+	+	+
7		+	+	+	+
8		−	+	+	+
9	Yogurt	−	+	+	+
10		+	+	+	+

+: Positive; -: Negative.

The European Federation of Animal Science (EFAS) and the European Food Safety Authority (EFSA) recommend that probiotics which are utilized in several food and pharmaceutics, should not have transferable antibiotic resistance genes to be considered safe for animal and human consumption (EFSA, 2007).

Antimicrobial potential is one of the crucial aspects to assess the probiotic properties of a microbe (Lashani, Davoodabadi, & Dallal, 2020; Montassier et al., 2021). The antibacterial activity of probiotics can be due to the synthesis of ethanol, diacetyl, proteins, phenols, H_2O_2, and organic acids like lactic and acetic acids that are formed during the probiotic's growth. These metabolites along with assistance of a competitive exclusion mode of action, in which probiotics compete with harmful bacteria for adhesive nutrients and receptors, can inhibit the pathogen's colonization in the human (Aditya, Peng, Young, & Biswas, 2020).

Lactic acid bacteria (LAB) could produce metabolites which affect the growth of pathogens (Ali et al., 2019). CFS prepared from some LAB reported to inhibit the growth of *B. cereus* strains (Khiralla, Mohamed, & Elhariry, 2015; Mahasneh, Hamdan, & Mahasneh, 2015; Soria & Audisio, 2014). Data in the current study (Table 3, Fig. 2) showed that *L. plantarum* CFS has antibacterial activity not only against *B. cereus* isolated from Kareish cheese and yogurt samples but also against *Bacillus cereus* EMCC1006 reference strain.

Different concentrations of *L. plantarum* CFS (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL) were used to detect minimum inhibitory concentrations (MICs) affecting the growth of *B. cereus* EMCC1006. As shown in Table 4, the inhibition zones of CFS of *L. plantarum* were high with the highest concentrations. MICs of *L. plantarum* CFS against *B. cereus* EMCC1006 reference strain was 3.1 mg/mL. These results were dissimilar to that reported by (Yusra & Likaa, 2013) who found that MIC of *L. plantarum* CFS against *B. cereus* cheese isolates was 0.07 mL.

Indeed, several recent reports confirmed that probiotic's utilization is considered as one of the biological based anti-microbial approaches that could effectively inhibit the growth of pathogenic microorganisms such as *L. monocytogenes* (Wu et al., 2022); another research reported that both the coaggregation impact and the cell free supernatant activity of *L. casei* IMAU60214 make this bacterium promising candidate for use as a probiotic with potential to interfere with the activity of some of the pathogenic factors of diarrheagenic *E. coli* strains (Rocha-Ramírez, Hernández-Chiñas, Moreno-Guerrero, Ramírez-Pacheco, & Eslava, 2023). Likewise, *Lactobacilli* strains such as *L. fermentum* LBF433 and *L. casei* LBC 237 have antimicrobial activity against *Salmonella* spp. and thus, demonstrated the probiotic potential (Lando, Valduga, & Moroni, 2023).

Lactic acid bacteria i.e., *L. rhamnosus*, *L. plantarum*, *L. fermentum*, *L. paracasei*, *L. casei*, *Lactobacillus* sp., *Enterococcus faecalis*, *E. faecium* (1.8%), and *E. durans* exhibited broad-spectrum antimicrobial characteristics against foodborne pathogens (Haryani et al., 2023). Therefore, lactic acid bacteria play a significant role in preservation the fermented dairy products (Angmo, Kumari, & Bhalla, 2016).

Our results showed that CFS of *L. plantarum* had a very good inhibitory effect on the *B. cereus* foodborne pathogens. In sum, *L. planetarium* CFS showed good probiotic properties such as antimicrobial activity against foodborne *B. cereus* pathogens in some investigated dairy foods i.e., Kareish cheese and yogurt.

3.4. Antioxidant potential and phenolic compounds of L. plantarum CFS

Phenolic compounds of *L. plantarum* CFS (μg/mL) were analyzed using HPLC. As shown in Table 5, some phenolic substances were higher percentage and other molecules recorded a low ratio. Probiotics supernatant shows various phenolic molecules such as salicylic acid and benzoic acid which may be responsible for antibacterial activity of *L. plantarum* CFS as well antioxidant potential. Moreover, gallic acid plays an important role in protective the probiotics from fungi attacking.

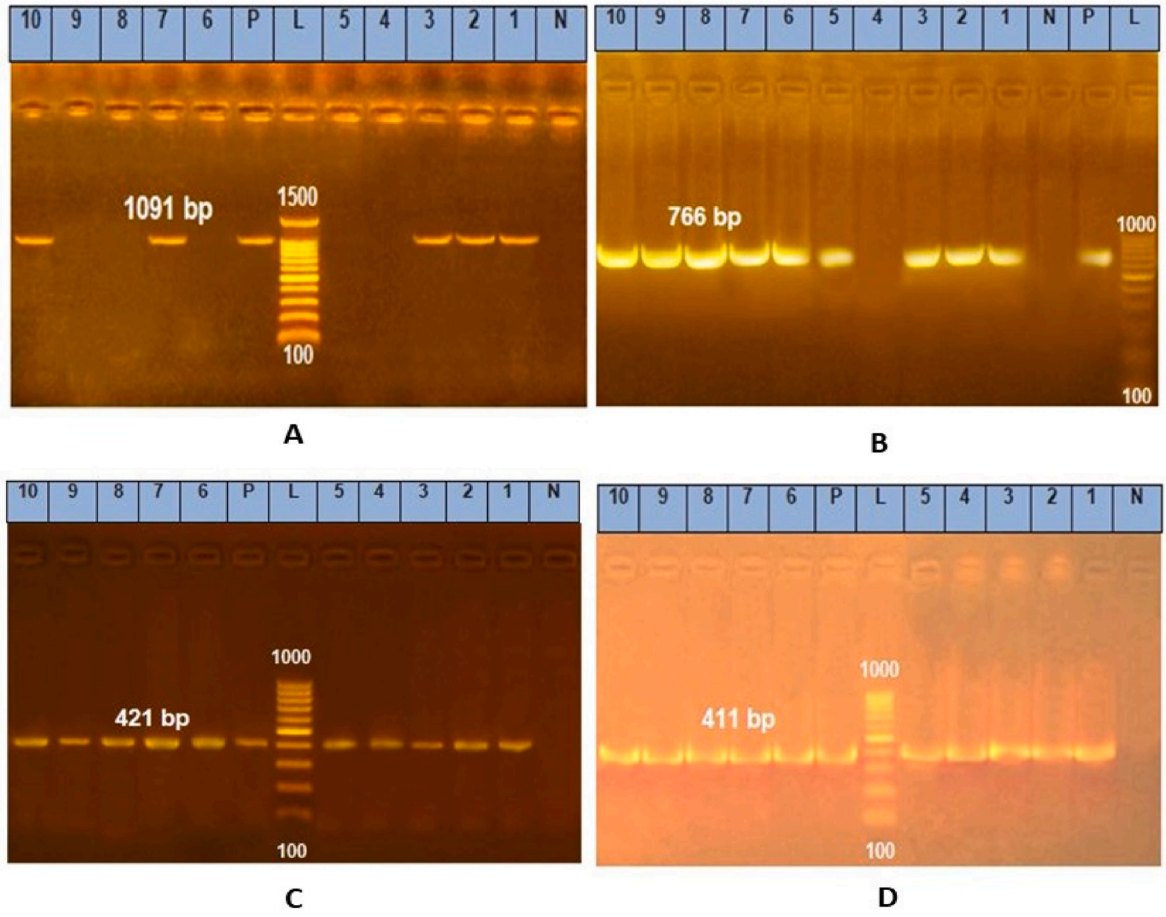

Fig. 1. (A) Agarose gel electrophoresis of PCR products of *Bacillus cereus* genes found in examined samples by PCR. *hbl* (1091bp), lanes (1- 10) are *Bacillus cereus* isolates, and lane (L) is DNA ladder 100 bp molecular weight marker, lane (N) is negative control, and lane (P) is positive control. **(B)** Agarose gel electrophoresis of PCR products of *Bacillus cereus* genes found in examined samples by PCR. *nhe* (766bp), lanes (1- 10) are *Bacillus cereus* isolates, and lane (L) is DNA ladder 100 bp molecular weight marker, lane (N) is negative control, and lane (P) is positive control. **(C)** Agarose gel electrophoresis of PCR products of *Bacillus cereus* genes found in examined samples by PCR. *cytk* (421bp), lanes (1- 10) are *Bacillus cereus* isolates, and lane (L) is DNA ladder 100 bp molecular weight marker, lane(N) is negative control, and lane (P) is positive control. **(D)** Agarose gel electrophoresis of PCR products of *Bacillus cereus* genes found in examined samples by PCR. *Pc-plc (411bp),* lanes (1- 10) are *Bacillus cereus* isolates, and lane (L) is DNA ladder 100 bp molecular weight marker, lane (N) is negative control, and lane (P) is positive control.

Table 2
Antibacterial susceptibility pattern of *B. cereus* isolates (n = 10).

Antibacterial agents	MIC concentrations interpretive (μg/mL) [a]			Results
	Sensitive	Intermediate	Resistant	
Ciprofloxacin	≤1	2	≥4	Intermediate
Erythromycin	≤0.5	1–4	≥8	Sensitive
Gentamicin	≤4	8	≥16	Sensitive
Tetracycline	≤4	8	≥16	Resistant

[a] Serrano Cardona and Muñoz Mata (2013).

These findings are in parallel with Candela, Alcazar, Espin, Egea, and Almela (1995) who reported that polyphenols (mainly soluble fraction types) have diverse resistance to infection. The authors also reported that the most inhibitory impact was formed by T-cinnamic acid, p-hydroxy benzoic acid, salicylic acid, and vanillin. Moreover, Niku-Paavola, Laitila, Mattila-Sandholm, and Haikara (1999) conveyed that the bioactive phenolic substances i.e., p-benzoic acid in probiotics *L. plantarum* could inhibit growth of the tested organism by 40% alone.

Probiotics *Lactobacillus* spp. displayed high TPC and TFC, and thus, exhibited a potential to scavenge DPPH radicals based on the phenolic and flavonoid compounds (Talib et al., 2019). As shown in Table 5, TPC and TFC of *L. plantarum* CFS were 18.5 (μg GAE/g) and 2.67 (μg QE/g), respectively. Aouadhi, Maaroufi, and Mejri (2014) reported that TPC in

Table 3
Antibacterial activity of *L. plantarum* cell free supernatant (CFS) against *B. cereus* isolates (n = 1–10) and *B. cereus* EMCC1006. (n = 11 and 12).

B. cereus isolates No.	*B. cereus* isolates source	Inhibition zone diameter (mm)
1	Kareish cheese	2.4 ± 0.41^{d}
2		2.6 ± 0.35^{d}
3		1.9 ± 0.15^{e}
4		2.1 ± 0.23^{e}
5		2.5 ± 0.65^{d}
6		3.1 ± 0.84^{c}
7		2.3 ± 0.75^{d}
8		3.2 ± 0.61^{bc}
9	Yogurt	3.5 ± 0.55^{b}
10		2.9 ± 0.25^{c}
11	*B. cereus* EMCC1006*	4.2 ± 0.75^{a}
12		NZ

NZ: no zone; *Reference strain; Means in the same column with a different superscript letter are significantly different ($p < 0.05$).

Lactobacillus strains CFS ranged from 202.7 (μg GAE/g) to 283.4 (μg GAE/g) and the TFC value ranged from 22.26 (μg QE/g) to 56.60 (μg QE/g). Additionally, Xiao et al. (2015) found that *L. plantarum* can form high phenolic substances levels during fermentation process. These results were in contrast with that conducted by Seddiek, Hamad, Zeitoun, Zeitoun, and Ali (2020) who found that TPC of the plant extracts with

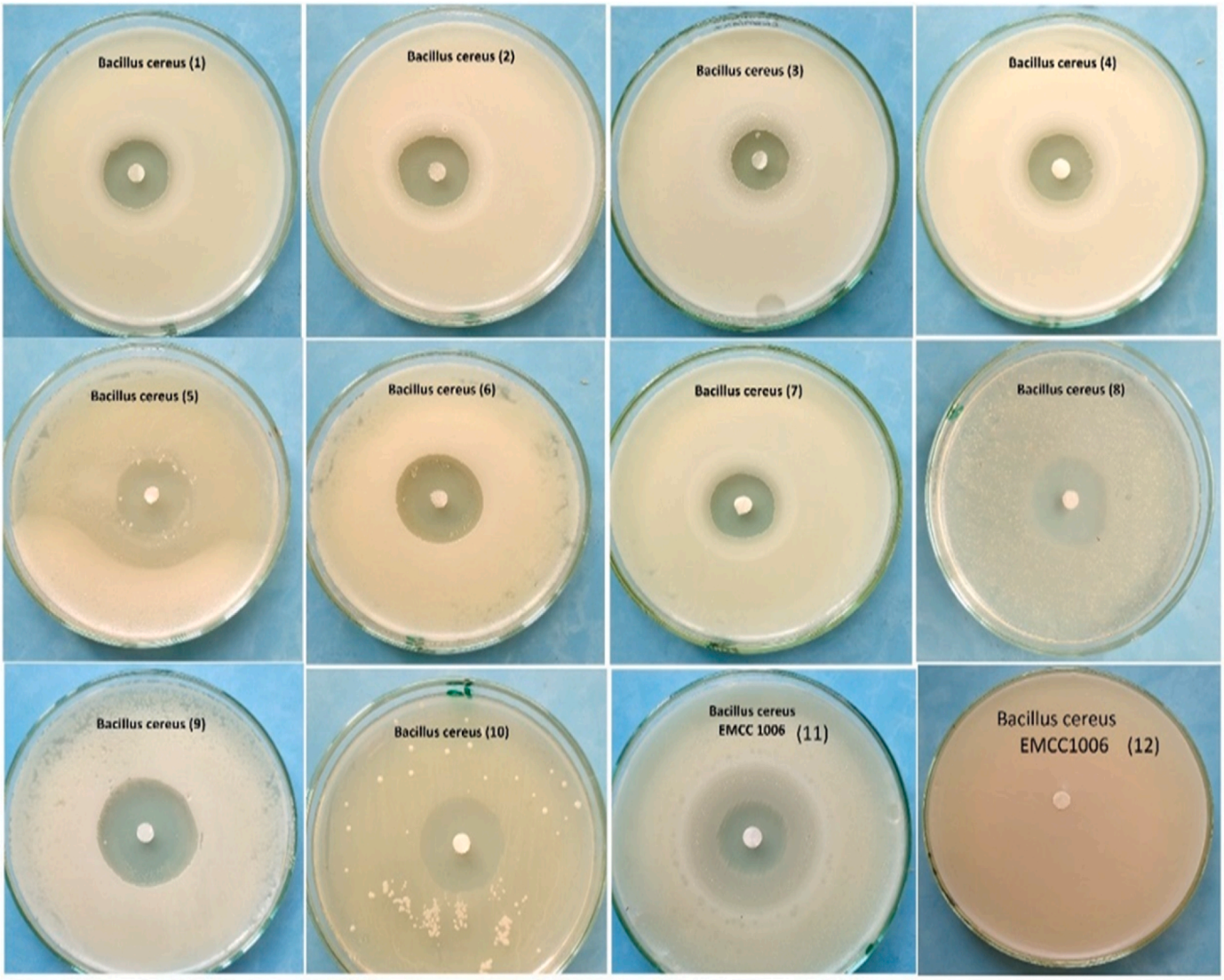

Fig. 2. Antibacterial activity of *L. planetarium* cell free supernatants against 12 *B. cereus* isolates and *B. cereus* EMCC1006 indicated as zone of inhibition.

Table 4
Effect of different concentrations of *L. plantarum* CFS on *B. cereus* EMCC1006 indicated as zone of inhibition for each concentration.

Concentrations of *L. plantarum* CFS (mg/mL)	*B. cereus* EMCC1006 (zone of inhibition)
1.5	ND
3.1	11 ± 0.25^{f}
6.25	14 ± 0.44^{e}
12.5	17 ± 0.60^{d}
25	19 ± 0.94^{c}
50	24 ± 1.22^{b}
100	29 ± 1.75^{a}

ND: Not detected; Means in the same column with a different superscript letter are significantly different ($p < 0.05$).

antibacterial effects fluctuated from 48.08 to 324.08 mg/g, while TFC fluctuated from 11.53 to 65.85 mg/g.

Lactic acid bacteria particularly lactobacilli, have antioxidant effect which help human body to get rid of free radicals (Aouadhi et al., 2014; Bharti, Mehta, Mourya, & Ahirwal, 2018). Antioxidant activity of *L. plantarum* CFS was expressed as IC_{50}. As illustrated in Table 6, the IC_{50} of L-ascorbic acid, as the positive control, was 26.36 μg/mL. The IC_{50} value of *L. plantarum* CFS was 53.84 μg/mL. These findings higher than

Table 5
Biophenolic compounds of *L. plantarum* CFS (μg/mL) using HPLC, total phenolic contents (μg GAE/g), and total flavonoid contents (μg QE/g).

Phenolic compounds	Concentration
Gallic acid (μg/mL)	1.62 ± 0.21
Catechol (μg/mL)	0.79 ± 0.18
Hydroxy benzoic acid (μg/mL)	18.14 ± 1.23
Caffeine (μg/mL)	0.35 ± 0.07
Vanillic acid (μg/mL)	0.62 ± 0.18
Caffeic acid (μg/mL)	0.08 ± 0.01
Syringic acid (μg/mL)	ND
Vanillin (μg/mL)	1.54 ± 0.18
Coumaric acid (μg/mL)	0.06 ± 0.02
Ferulic acid (μg/mL)	0.09 ± 0.01
Ellagic acid (μg/mL)	0.61 ± 0.04
Benzoic acid (μg/mL)	2.13 ± 0.29
Coumaric acid (μg/mL)	0.70 ± 0.13
Salicylic acid (μg/mL)	3.83 ± 0.54
Cinnamic acid (μg/mL)	0.38 ± 0.05
Total phenolic content (μg GAE/g)	18.5 ± 0.18
Total flavonoids content (μg QE/g)	2.67 ± 0.25

ND: Not detected.

Table 6
Antioxidant activity of *L. plantarum* CFS (IC50 μg/mL) using DPPH assay with ascorbic acid as standard.

Conc. (μg/mL)	Ascorbic acid		*L. plantarum* CFS	
	Inhibition (%)	IC_{50} (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
10	5.11 ± 0.28[a]	26.36 ± 0.14[a]	12.16 ± 0.33[b]	53.84 ± 0.21[b]
20	35.19 ± 2.21[a]		19.21 ± 1.17[b]	
30	56.89 ± 2.18[a]		28.43 ± 1.41[b]	
40	80 ± 1.78[a]		39.62 ± 1.74[b]	
50	89.61 ± 2.63[a]		46.51 ± 1.32[b]	
60	94.72 ± 2.38[a]		55.72 ± 0.98[b]	
70	97.20 ± 1.41[a]		73.25 ± 1.48[b]	
80	98.68 ± 1.03[a]		82.59 ± 1.71[b]	
90	99.34 ± 0.14[a]		91.82 ± 1.14[b]	
100	99.67 ± 0.10[a]		98.93 ± 0.08[a]	

Means in the same row with a different superscript letter are significantly different ($p < 0.05$).

that reported by Kim, Lee, Jeong, and Kang (2022) who reported that IC_{50} value of different LAB ranged from 2.55 to 6.88%. Analysis of DPPH free radical scavenging of *L. plantarum* IH14L revealed that 90.34% strain exhibited the highest activity (Düz, Doğan, & Doğan, 2020).

Moreover, *Lactobacilli* were found to be powerful as DPPH radical scavengers (Hamad, Ombarak, et al., 2022). This result agrees with that obtained by Kocak, Sanli, Anli, and Hayaloglu (2020), who found that the highest antioxidant power was allied to the aqueous extract of *L. bulgaricus* in ripened feta cheese compared to extracts of *L. paracasei* or *L. plantarum*. The study of Yousefi, Dovom, Najafi, and Mortazavian (2021) reported that the highest DPPH radical scavenging of *L. brevis* in the supernatant of ripened cheese was 31.45%. Indeed, the potential power and biologically role of phenolic molecules for human health as antioxidant, antibacterial, anti-inflammatory, anticancer, and many health benefits were demonstrated (Ali et al., 2022; Mehany et al., 2021).

3.5. Sensory evaluation of kareish cheese and yogurt samples mixed with several ratios of L. plantarum CFS

One way of delivering a probiotic into the human body is via the incorporation of the probiotic into food. Kareish cheese and yogurt samples were mixed with various ratios of *L. plantarum* CFS (1.5, 3.1, 6.25, 12.5, 25.0, 50.0, 100.0 mg/mL) at the time of preparation and the sensory properties were evaluated. As shown in Table 7, the sensory attributes of both treated Kareish cheese and yogurt samples scored excellent at the lowest concentrations (1.5 and 3.1 mg/mL of *L. plantarum* CFS). At concentration 6.25, 12.5 and 25 mg/mL CFS, sensory properties scored very, very good. While, at concentration 50 and 100 mg/mL CFS, sensory properties have a very good score. These results indicate the valuable effect of *L. plantarum* CFS on the sensory properties of Kareish cheese and yogurt in addition to its antimicrobial and antioxidant effects. The low sensorial acceptance of high probiotic ratio is may be due to the low texture attributes in the fortified products (less homogenous).

Our findings are in parallel with those reported by Ngamsomchat et al. (2022), the authors concluded that textures of the chèvre cheese fortified by *L. plantarum* AD73 seemed to be affected by the incorporation of the probiotic, which caused the cheese to be less homogenous and drier owing to the lumpiness in the cheese curd, and thus, may be related to the moisture. On the other hand, the overall texture of the probiotic cheese was still acceptable. The authors also indicated that *L. plantarum* AD73 could be used to produce a novel probiotic chèvre cheese due to its antibacterial power against foodborne pathogens and its capability to ferment a wide variety of carbohydrates that support its probiotic potential.

The results of the present study provide critical information for understanding the potential role of probiotic as an antimicrobial and antioxidant candidate to produce a healthy dairy food, low/free of *B. cereus,* with good organoleptic properties. The outcomes of this study will lead future direction of using of *L. planetarium* cell free supernatant in dairy industry.

4. Conclusions

Contamination of Kareish cheese and yogurt with virulent *B. cereus* has a great public health concern. Therefore, it is necessary to track the hygienic and sanitation levels throughout handling chain and processing of these dairy products to overcome this foodborne pathogen. Moreover, the current findings confirm that incorporation of *L. plantarum* cell free supernatant to Kareish cheese and yogurt can prevent the development of pathogenic *B. cereus* thanks to its antimicrobial and antioxidant potential. The antibacterial effect of *L. plantarum* CFS is dose dependent, finally, the lower concentrations of *L. plantarum* CFS improved the sensory properties of Kareish cheese and yogurt samples.

Credit author statement

Walaa I. Ahmed: Formal analysis, Validation, Investigation, Resources. **Ayman. M. Kamar**: Formal analysis, Investigation, Resources. **Gamal M. Hamad**: Conceptualization, Methodology, Software, Writing – original draft, Visualization, Supervision. **Taha Mehany**: Conceptualization, Formal analysis, Data curation, Investigation, Validation, Software, Writing – original draft, Writing – review & editing, Language editing and proofing. **Wahid I. El-Desoki**: Visualization, Validation, Investigation. **Eman Ali**: Software. **Jesus Simal-Gandara**: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 7
Sensorial attributes of Kareish cheese and yogurt samples prepared with different concentrations of *L. planetarium* CFS.

Sample	Concentrations of *L. plantarum* CFS mg/mL						
	1.5	3.1	6.25	12.5	25	50	100
Kareish cheese	Excellent	Excellent	Very, Very Good	Very, Very Good	Very, Very Good	Very Good	Very Good
Yogurt	Excellent	Excellent	Very, Very Good	Very, Very Good	Very, Very Good	Very Good	Very Good

the work reported in this paper

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114946.

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