Original Article
Effect of Adding Lactoferrin on Some Foodborne Pathogens in Yogurt

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ABSTRACT

Background: Lactoferrin is a natural biological active cationic protein that can be used as a yogurt additive to inhibit the growth of foodborne pathogens.

Objectives: The present study evaluated the antimicrobial effects of lactoferrin against Bacillus cereus, Enterococcus faecalis, and Candida albicans inoculated in laboratory prepared yogurt at refrigerator temperature.

Methods: The pre-warmed skimmed milk was inoculated by commercial starter and then divided into 3 parts, each inoculated by different inoculum and a certain concentration of lactoferrin (0.5% and 1.5%) and incubated at 42°C for 4 h till curd formation. Tenfold serial dilutions were performed for each group and refrigerated at 4±1°C for up to 14 days to be examined every day.

Results: The obtained results showed that the treated yogurt samples with lactoferrin had significant reductions in B. cereus, Ent. faecalis, and C. albicans counts than untreated samples (control positive). Generally, yogurt samples treated with 1.5% lactoferrin showed the highest reduction percentages on B. cereus and C. albicans than 0.5% lactoferrin. In addition, B. cereus showed more susceptibility to lactoferrin than Ent. faecalis; the maximum reduction of the inoculated B. cereus was observed on the sixth day of the incubation to be 99.99%. Maximum reduction of the tested C. albicans was observed after the seventh day.

Conclusion: The application of lactoferrin showed a potentially significant antimicrobial effect against B. cereus, Ent. faecalis, and C. albicans in refrigerating conditions, so lactoferrin is recommended to be used in yogurt production for safe product manufacturing.

Keywords: Yogurt, Lactoferrin, Bacillus cereus, Enterococcus faecalis, Candida albicans
1. Introduction

Yogurt is a fermented dairy product popular among people in Egypt and worldwide. It is a rich source of minerals such as calcium, proteins, fats, and useful microorganisms such as *Streptococcus thermophiles* and *Lactobacillus bulgaricus* (Alighazi et al., 2020). In recent years, scientists have tried to increase the organoleptic and health properties of yogurt using different methods (Fernandez & Marette, 2017).

Its production and consumption are growing continuously due to its health benefits, let alone its high nutritive value (El Kholy et al., 2014). Also, fortifying yogurt with lactoferrin can add more health benefits (Tomita et al., 2009; Tsukahara et al., 2020). However, yogurt is highly vulnerable to bacterial contamination, and hence it is easily perishable (Girma et al., 2014).

Factors affecting the hygienic quality of raw milk, especially mastitis, is an important disease that affects dairy herds and products around the world, which has mainly been caused by different pathogens, including *Staphylococcus aureus* (*S. aureus*). This pathogen tends to form biofilm and may be antibiotic resistance (Foroutan et al., 2022). The microbial quality of yogurt represents the quality of raw milk. Due to unsanitary conditions, there is a possibility of microbial contamination, which may have serious effects on the consumer’s health (Qajarbeygi et al., 2017).

Lactoferrin is a glycoprotein that can bind and transfer iron; it is found in many secretions in the body as saliva, serum, and tears, and is highly present in milk and colostrum. Lactic fermentation of foods, such as yogurt, increases the availability of iron (Yen et al., 2011; Lisko et al., 2017).

Lactoferrin is known to be a multifunctional or multi-tasking protein. It has antimicrobial, antioxidant, anti-cancer, anti-inflammatory, and immune modulators, and it represents an excellent natural alternative substance that reduces the use of chemical preservatives (Ochoa & Cleary, 2009; Legrand, 2016). So, the present study was planned to determine the antimicrobial effects of LF on the viability of some pathogenic microbes, *Bacillus cereus*, *Enterococcus faecalis*, and *Candida albicans* strains in yogurt.

2. Materials and Methods

Bovine lactoferrin (LF) was purchased from Hygint pharmaceuticals, Alexandria, Egypt. It was prepared using sterile distilled water to obtain LF solution at concentrations of 0.5% and 1.5% (Ombarak et al., 2019). LF is the ideal compound of choice due to its stability and supply of high iron bioavailability, and it does not affect the sensory properties or nutritional value of yogurt (El-Kholy et al., 2011).

Activation of yogurt starter cultures

Lyophilized mixed starter cultures containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) were obtained from the Cairo MIR-CEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University. Lyophilized mixed starter cultures were added to sterile 11% reconstituted skimmed milk powder and incubated at 37°C for 24 h (Tavakoli et al., 2019).

Inoculum preparation

Using natural antimicrobial compounds such as lactoferrin in food has gained much attention from consumers and the food industry. This interest is primarily due to two major factors. First, the misuse and mishandling of antibiotics have resulted in the dramatic rise of a group of microorganisms, including foodborne pathogens, that are not only antibiotic-resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers’ awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. This condition has prompted the food industry to look for alternative preservatives that can enhance the safety and quality of foods. Compounds derived from natural sources, such as lactoferrin (LF), can be used for food safety due to their antimicrobial properties against a broad range of foodborne pathogens (Tajkarimi et al., 2010; Gyawali & Ibrahim, 2014; Niaz et al., 2019).
Preparations of yoghurt

The skim milk was heated to 85°C for 30 min and immediately cooled to 45°C, and then inoculated with the activated starter cultures (Corrieu & Be’al., 2016), followed by the addition of the examined pathogen and LF. Samples were grouped as follows:

**B. cereus groups**

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^6 CFU/mL *B. cereus* (control positive).

G3: Yogurt+10^6 CFU/mL *B. cereus*+0.5% LF.

G4: Yogurt+10^6 CFU/mL *B. cereus*+1.5% LF.

**E. faecalis Groups**

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^6 CFU/mL *E. faecalis* (control positive).

G3: Yogurt+10^6 CFU/mL *E. faecalis*+0.5% LF.

G4: Yogurt+10^6 CFU/mL *E. faecalis*+1.5% LF.

**C. albicans Groups**

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^3 CFU/mL *C. albicans* (control positive).

G3: Yogurt+10^3 CFU/mL *C. albicans*+0.5% LF.

G4: Yogurt+10^3 CFU/mL *C. albicans*+1.5% LF.

All samples were packed into sterile polyethylene cups, labeled, and incubated at 44°C till curd formation, then stored at 4°C for 14 days and examined every day to monitor *B. cereus*, *E. faecalis*, and *C. albicans* counts (FDA, 2001; Domig et al., 2003 & ISO, 2008, respectively). Tests were performed in triplicate.

Microbiological examinations

**Preparation of samples**

It was performed according to ISO (2017).

Counting of inoculated pathogens

*B. cereus* counting was performed on *B. cereus* agar (FDA, 2001), while *E. faecalis* counting was performed on KF Streptococcus agar (Domig et al., 2003), and *C. albicans* counting on modified Sabouraud dextrose agar (ISO, 2008).

Statistical analysis

The experiment for studying the effect of different lactoferrin concentrations on some isolates was conducted in three repetitions. Data were coded, then entered and analyzed using the SPSS software, version 26-2018 (SPSS; IBM Corp, NY, USA) for Microsoft Windows 10.

3. Results

The antibacterial activity of Lactoferrin on the viability of *B. cereus* in yogurt

The antibacterial activity of LF on the viability of experimentally inoculated pathogenic *B. cereus* strains in yogurt is shown in Table 1. It was revealed that treated groups (0.5% and 1.5% LF) showed lower *B. cereus* counts than the control group, where the mean *B. cereus* counts in examined yogurt samples after the addition of 0.5% LF were lowered from 4.2×10^6±0.06×10^6 on day 0 to 1.2×10^2±0.03×10^2 on day 14 of storage with reduction percentage from 67.56% to 99.99% on the first and fourteen days, respectively. In the case of using 1.5% LF, the mean counts were lowered from 4.2×10^6±0.06×10^6 on day 0 to 1.2×10^2±0.12×10^2 on day 9 of inoculation with a reduction percentage from 90.81% to 99.99% on the first and ninth days, respectively, but not detected after that (<10^2 CFU/g) with 100% reduction percentage after the ninth day, comparing to *B. cereus* counts in the control samples which were increased from 4.2×10^6±0.06×10^6 on day 0 to 9.6×10^9±0.05×10^9 on day 9 of storage. In addition, all results showed a significant (P<0.05) decrease (growth inhibition) in *B. cereus* in the yogurt samples treated with 1.5% LF and 0.5% LF during refrigerated storage when compared with non-treated samples.

The antibacterial activity of LF on the viability of *E. faecalis* strain

Table 2 presents the antibacterial activity of LF on the viability of experimentally inoculated pathogenic *E. faecalis* strains in yogurt samples. The treated groups showed lower *E. faecalis* counts than the control groups, where the mean *E. faecalis* counts in examined yogurt samples after the addition of 0.5% LF decreased from
5.9 × 10⁶ ± 0.03 × 10⁶ on day 0 to 1.9 × 10⁶ ± 0.03 × 10⁶ on day 14 of storage with reduction percentage from 23.3% to 99.9% on the first and ninth days, respectively. In the case of using 1.5% LF, the mean counts decreased from 5.9 × 10⁶ ± 0.03 × 10⁶ on day 0 to 1.3 × 10⁵ ± 0.01 × 10⁵ on day 14 of storage with a reduction percentage from 27.4% to 99.9% on first and ninth days, respectively, comparing to *E. faecalis* counts in the control samples which it increased from 5.9 × 10⁶ ± 0.03 × 10⁶ on day 0 to 9.1 × 10⁹ ± 0.06 × 10⁹ on day 10 of storage. Moreover, all results showed a significant (P<0.05) decrease (growth inhibition) of *E. faecalis* in the yogurt samples treated with 0.5% and 1.5% LF during cold storage when compared with non-treated samples.

### The antifungal activity of LF on the viability of *C. albicans* in yogurt

The antifungal activity of LF on the viability of experimentally inoculated pathogenic *C. albicans* strains in yogurt is shown in Table 3. The treated groups showed lower *C. albicans* counts than the control group, where the mean *C. albicans* counts in examined yogurt samples after the addition of 0.5% LF decreased from 1.5 × 10⁹ ± 0.03 × 10⁹ on day 0 to 4.0 × 10⁸ ± 0.003 × 10⁸ on day 11 of storage with reduction percentage from 4.76% to 99.99% at the first and eleventh days, respectively, but not detected after that (<10⁵) with 100% reduction percentage. In the case of using 1.5% LF, the mean counts decreased from 1.5 × 10⁹ ± 0.04 × 10⁹ on day 0 to 9.6 × 10⁶ ± 0.008 × 10⁶ on day 10 of inoculation, with a reduction percentage from 23.81% to 99.9% on the first and ninth days, respectively, but not detected after that (<10⁵) with 100% reduction percentage, comparing to *C. albicans* counts in the control samples which increased from 1.5 × 10⁹ ± 0.03 × 10⁹ on day 0 to 9.1 × 10⁹ ± 0.01 × 10⁹ on day 10 of inoculation, but not detected after that as the samples were spoiled. Moreover, all results showed a significant (P<0.05) decrease (growth inhibition) of *C. albicans* in the yogurt samples treated with 0.5% and 1.5% LF during cold storage when compared with non-treated samples.

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**Table 1.** Antibacterial activity of lactoferrin on viability of *Bacillus cereus* inoculated into yogurt during refrigeration storage (4°C)

<table>
<thead>
<tr>
<th>Storage Time (Day)</th>
<th>Control Group Negative</th>
<th>Control Group Positive 0.5% Lactoferrin Group</th>
<th>1.5% Lactoferrin Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>Mean±SEM R%</td>
<td>Mean±SEM R%</td>
</tr>
<tr>
<td>0</td>
<td>4.2×10⁶±0.06×10⁶</td>
<td>4.2×10⁶±0.06×10⁶ --</td>
<td>4.2×10⁶±0.06×10⁶ --</td>
</tr>
<tr>
<td>1</td>
<td>7.4×10⁶±0.01×10⁶</td>
<td>2.4×10⁶±0.06×10⁶ 67.56</td>
<td>6.8×10⁶±0.06×10⁶ 90.81</td>
</tr>
<tr>
<td>2</td>
<td>9.8×10⁵±0.06×10⁵</td>
<td>3.7×10⁵±0.03×10⁵ 96.2</td>
<td>1.1×10⁵±0.03×10⁵ 98.8</td>
</tr>
<tr>
<td>3</td>
<td>1.1×10⁵±0.01×10⁵</td>
<td>3.1×10⁵±0.03×10⁵ 97.18</td>
<td>7.2×10⁵±0.06×10⁵ 99.3</td>
</tr>
<tr>
<td>4</td>
<td>2.1×10⁵±0.07×10⁵</td>
<td>9.0×10⁴±0.03×10⁴ 99.57</td>
<td>2.8×10⁴±0.08×10⁴ 99.86</td>
</tr>
<tr>
<td>5</td>
<td>3.7×10⁴±0.02×10⁴</td>
<td>5.5×10³±0.03×10³ 99.85</td>
<td>8.5×10²±0.01×10² 99.97</td>
</tr>
<tr>
<td>6</td>
<td>5.1×10³±0.04×10³</td>
<td>3.4×10²±0.03×10² 99.99</td>
<td>4.3×10¹±0.002×10¹ 99.99</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>9.4×10⁴±0.03×10⁴ 99.99</td>
<td>9.6×10³±0.008×10³ 99.99</td>
</tr>
<tr>
<td>8</td>
<td>1.9×10³±0.06×10³</td>
<td>7.7×10²±0.06×10² 99.99</td>
<td>5.4×10¹±0.005×10¹ 99.99</td>
</tr>
<tr>
<td>9</td>
<td>9.6×10²±0.05×10²</td>
<td>4.2×10¹±0.03×10¹ 99.99</td>
<td>1.2×10⁰±0.10×10⁰ 99.99</td>
</tr>
<tr>
<td>10</td>
<td>S</td>
<td>1.3×10⁰±0.05×10⁰ &lt;10⁰</td>
<td>&lt;10⁰</td>
</tr>
<tr>
<td>11</td>
<td>S</td>
<td>9.3×10⁰±0.03×10⁰ &lt;10⁰</td>
<td>&lt;10⁰</td>
</tr>
<tr>
<td>12</td>
<td>S</td>
<td>8.9×10⁰±0.05×10⁰ &lt;10⁰</td>
<td>&lt;10⁰</td>
</tr>
<tr>
<td>13</td>
<td>S</td>
<td>9.1×10⁰±0.01×10⁰ &lt;10⁰</td>
<td>&lt;10⁰</td>
</tr>
<tr>
<td>14</td>
<td>S</td>
<td>1.2×10⁰±0.03×10⁰ &lt;10⁰</td>
<td>&lt;10⁰</td>
</tr>
</tbody>
</table>

a, b, c, ab Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviations: S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of *B. cereus* count.
Lactoferrin is a natural component used as a food additive to inhibit the growth of pathogenic microorganisms (Ombarak et al., 2019). It is a protein that occurs naturally in milk and nowadays is increasingly supplemented in foods for its multiple functions and its antimicrobial effects on many bacteria and yeast (Zorina et al., 2018 & Niaz et al., 2019). Lactoferrin is essential to produce a supplemented yogurt that could alleviate symptoms caused by some gastrointestinal problems (Bruni et al., 2016; Zarzosa-Moreno et al., 2020).

Concerning the antibacterial effect of LF on *B. cereus* counts in yogurt samples, the results showed that the control samples had the highest counts of *B. cereus* at cold storage compared to other treatments, and also, yogurt samples treated with 1.5% LF had the highest reduction percentage of *B. cereus* counts than 0.5% LF (Table 1). These results agree with those of (Karam-Allah, 2022), who recorded that lactoferrin has a strong inhibitory effect against *B. cereus* by using LF at a concentration of 100 mg/g in stirred yogurt. In addition, Ombarak et al., (2019) recorded that 4% LF inhibited *B. cereus* strains and decreased their counts in experimentally inoculated Kareish cheese.

The mechanism of LF action is the direct interaction between the positively charged protein regions with anionic molecules present on the surface of some microorganisms, resulting in increased membrane permeability that leads to bacterial and fungal damage (Haversen et al., 2010).

Lactoferrin has antimicrobial activities for many pathogenic microbes, including Enteropathogenic *E. coli*, *E. faecalis*, *B. cereus*, and *C. albicans* (Farnaud & Evans, 2003; Pan et al., 2007).

Lactoferrin’s antibacterial activity on Gram-positive bacteria is attributed to its binding to iron, leading to the inhibition of bacterial growth via restriction of the avail-

### Table 2. Antibacterial activity of lactoferrin on viability of *E. faecalis* inoculated into yogurt during their refrigeration storage (4°C)

<table>
<thead>
<tr>
<th>Storage Time (Day)</th>
<th>Control Group Negative</th>
<th>0.5% Lactoferrin Group</th>
<th>1.5% Lactoferrin Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>R%</td>
</tr>
<tr>
<td>0</td>
<td>5.9x10^6±0.3x10^6</td>
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<td>--</td>
</tr>
<tr>
<td>1</td>
<td>7.3x10^6±0.1x10^6</td>
<td>5.6x10^6±0.06x10^6</td>
<td>23.3</td>
</tr>
<tr>
<td>2</td>
<td>9.5x10^6±0.06x10^6</td>
<td>5.3x10^6±0.03x10^6</td>
<td>44.2</td>
</tr>
<tr>
<td>3</td>
<td>2.6x10^6±0.06x10^6</td>
<td>5.0x10^6±0.03x10^6</td>
<td>80.7</td>
</tr>
<tr>
<td>4</td>
<td>5.4x10^6±0.03x10^6</td>
<td>4.6x10^6±0.06x10^6</td>
<td>91.4</td>
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<tr>
<td>5</td>
<td>8.2x10^6±0.03x10^6</td>
<td>4.1x10^6±0.03x10^6</td>
<td>95.0</td>
</tr>
<tr>
<td>6</td>
<td>3.1x10^6±0.06x10^6</td>
<td>3.2x10^6±0.03x10^6</td>
<td>98.9</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>8.1x10^6±0.03x10^6</td>
<td>99.7</td>
</tr>
<tr>
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<td>5.4x10^6±0.03x10^6</td>
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</tr>
<tr>
<td>10</td>
<td>S</td>
<td>5.7x10^6±0.06x10^6</td>
<td>99.9</td>
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<tr>
<td>11</td>
<td>S</td>
<td>4.5x10^6±0.03x10^6</td>
<td>99.9</td>
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<td>12</td>
<td>S</td>
<td>3.0x10^6±0.03x10^6</td>
<td>99.9</td>
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<tr>
<td>13</td>
<td>S</td>
<td>2.1x10^6±0.03x10^5</td>
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<tr>
<td>14</td>
<td>S</td>
<td>1.9x10^6±0.03x10^5</td>
<td>99.9</td>
</tr>
</tbody>
</table>

*a, b, c, ab Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviation: ND: Normal Data; S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of *E. faecalis* count.

### 4. Discussion

Lactoferrin is a natural component used as a food additive to inhibit the growth of pathogenic microorganisms (Ombarak et al., 2019). It is a protein that occurs naturally in milk and nowadays is increasingly supplemented in foods for its multiple functions and its antimicrobial effects on many bacteria and yeast (Zorina et al., 2018 & Niaz et al., 2019). Lactoferrin is essential to produce a supplemented yogurt that could alleviate symptoms caused by some gastrointestinal problems (Bruni et al., 2016; Zarzosa-Moreno et al., 2020).

The mechanism of LF action is the direct interaction between the positively charged protein regions with anionic molecules present on the surface of some microorganisms, resulting in increased membrane permeability that leads to bacterial and fungal damage (Haversen et al., 2010).

Lactoferrin has antimicrobial activities for many pathogenic microbes, including Enteropathogenic *E. coli*, *E. faecalis*, *B. cereus*, and *C. albicans* (Farnaud & Evans, 2003; Pan et al., 2007).

ability of iron as a nutrient for bacteria, and to its effects on lipoteichoic and teichoic acids, which leads to depolarization and disruption of bacterial membranes than on the cytoplasmic contents (Brandenburg et al., 2001; Orsi, 2004; Liu et al., 2011).

Moreover, lactoferrin can counter different important mechanisms evolved by microbial pathogens to infect and invade the host, such as adherence, colonization, invasion, and production of biofilms, and also cause mitochondrial and caspase-dependent regulated cell death and apoptosis-like in pathogenic yeasts (Yen et al., 2011; Sharbfi et al., 2016; Zarzosa-Moreno et al., 2020). Moreover, regarding the antifungal effect of lactoferrin on C. albicans counts in yogurt samples, the obtained results showed that the treated groups had much lower counts (growth inhibition) compared with the control ones. Also, yogurt samples treated with 1.5% lactoferrin had the highest reduction percentage of C. albicans counts than 0.5% lactoferrin (Table 3). C. albicans was found to be highly susceptible to inhibition and inactivation by concentration within the 18 to 150 μg/mL range (Bellamy et al., 1993); lactoferrin concentration (20 μg/mL) caused a rapid loss of viability of C. albicans isolates (Samaranayake et al., 2001). The concentration of lactoferrin was required for the suppression of C. albicans (11.3±1.5 and 43.8±9.5 pg/mL) (Zorina et al., 2018).

Table 3. Antifungal activity of lactoferrin on viability of C. albicans inoculated into yogurt during their refrigeration storage (4°C)

<table>
<thead>
<tr>
<th>Storage Time (Day)</th>
<th>Control Group Positive</th>
<th>0.5% Lactoferrin Group</th>
<th>1.5% Lactoferrin Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>R%</td>
<td>Mean±SEM</td>
</tr>
<tr>
<td>0</td>
<td>15x10^7±0.3x10^4</td>
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<td>6</td>
<td>61x10^7±0.05x10^4</td>
<td>99.31</td>
<td>8.0x10^7±0.03x10^4</td>
</tr>
<tr>
<td>7</td>
<td>88x10^7±0.05x10^4</td>
<td>99.97</td>
<td>7.0x10^7±0.03x10^4</td>
</tr>
<tr>
<td>8</td>
<td>37x10^7±0.05x10^4</td>
<td>99.97</td>
<td>5.0x10^7±0.03x10^4</td>
</tr>
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<td>55x10^7±0.05x10^4</td>
<td>99.99</td>
<td>1.0x10^7±0.03x10^4</td>
</tr>
<tr>
<td>10</td>
<td>91x10^7±0.06x10^4</td>
<td>99.99</td>
<td>&lt;10^9</td>
</tr>
<tr>
<td>11</td>
<td>S</td>
<td>4.0x10^7±0.03x10^4</td>
<td>&lt;10^9</td>
</tr>
<tr>
<td>12</td>
<td>S</td>
<td>&lt;10^9</td>
<td>&lt;10^9</td>
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<td>14</td>
<td>S</td>
<td>&lt;10^9</td>
<td>&lt;10^9</td>
</tr>
</tbody>
</table>

a, b, c Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviations: S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of C. albicans count.
through sequestering Fe^{3+} ions, thinning and inhibiting hyphal development, altering the permeability of the cell surface, significantly preventing biofilms and decreasing the internal thiol levels with 20% in *C. albicans*, resulting in the death of cells.

According to the present study, we concluded that lactoferrin had highly antibacterial against *B. cereus* than *E. faecalis* which showed more resistance to it, so we need a higher concentration. Lactoferrin has antifungal effects on *C. albicans*, and naturally-occurring antimicrobials such as lactoferrin for yogurt preservation are gaining great attention due to consumers’ trends and help to reduce the addition of chemical preservatives.

**Ethical Considerations**

**Compliance with ethical guidelines**

There were no ethical considerations to be considered in this research.

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**Authors’ contributions**

All authors equally contributed to preparing this article.

**Conflict of interest**

The authors declared no conflict of interest.

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**References**


