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ORIGINAL ARTICLE

# A survey on biofilm formation of *Lactobacillus rhamnosus* (PTCC 1637) and *Lactobacillus plantarum* (PTCC 1745) as a survival strategy of probiotics against antibiotic in vitro and yogurt

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## Abstract

Probiotics are useful microorganisms with health effects. Although the probiotic industry has grown dramatically over the past decade, their survival is still challenging. Also, the residual of antibiotics is considered a serious problem with major health and technological problems in the fermented food industry. In this study, the biofilm technique was examined as a practical solution for increasing viability. The biofilm of *Lactobacillus rhamnosus* and *Lactobacillus plantarum* are formed in the culture medium (using microtiter plate biofilm method) and yogurt (in containers). The antibiotic susceptibility of probiotics to enrofloxacin, sulfadimidine, tetracycline, and oxytetracycline were studied. The results showed that enrofloxacin, the strongest antibiotic, reduces the bacterial population in the biofilm form only 2.6 log. In contrast, the population of bacteria reduced by about 8 log in plankton form. Therefore, biofilm techniques can be introduced as a survival strategy for the food and pharmaceutical industry.

## Practical applications

As a new and innovative approach, the biofilm method can lead to a new generation of probiotics, which can significantly protect probiotics against environmental stress and antibiotic residues and significantly affect their survival. The formation of biofilm by probiotics is a unique feature that is inherently bacterial and is a natural and low-cost method that can upgrade the long-term sustainability of probiotics. Therefore, biofilm can be commercially used to create new capacity in food and related industries.

## 1 | INTRODUCTION

Recently, the presence of antibiotic residues has been considered by researchers worldwide and has been raised as one of the most important challenges encountered (Baeza et al., 2016; Kjeldgaard et al., 2012). The World Health Organization (WHO), the American Medical Association, and the American Public Health Association called for a ban on antibiotics because these compounds cause various health problems in humans (Bacanlı & Başaran, 2019; Comunian et al., 2010). The FAO cites antibiotic residues as a threat and makes a unique effort to help governments, manufacturers, traders, and others to adopt facilities to minimize antibiotic use (FAO, 2016). Antibiotic residues caused significant challenges in various areas of human life, such as allergic reactions, bacterial resistance, disruption of the normal microflora of the gastrointestinal tract, carcinogenicity, mutations, and humans' malformations. It should be noted that ribosomes are the main target of most antibiotics. On the other hand, the presence of antibiotics in the food industry, especially among fermented foods such as meat and dairy products, cheese, and yogurt, causes adverse effects on starter culture and probiotic bacteria (Ashraf & Shah, 2011; Moghadam, 2016;

  

Movassagh & Karami, 2011; Nguyen et al., 2014; Rahman et al., 2021). In addition, with the development of the probiotic industry, having a large share of the global trade market, a new challenge has arisen that is the use of probiotics in products containing antibiotic residues because the most important issue facing probiotics is their survival, which is still challenging (Kellnerová et al., 2015; Mahendradatta et al., 2007; Mohan et al., 2020; Rowles, 2017; Shori et al., 2018). Probiotic survival is significant from several perspectives. First, survival during the product's storage and process; second, survival while passing through the gastrointestinal tract and inside the gastrointestinal tract; and third, survival against antibiotic residues (Shori et al., 2018). In the past few years, second- and third-generation probiotics have been developed through encapsulation and trapping bacteria in synthetic and natural polymer compounds to increase viability (Afzaal et al., 2019; Gul, 2017; Salas-Jara et al., 2016; Singh et al., 2019). Bacterial biofilm seems to be able to troubleshoot the survival problem because this phenomenon is a simple, convenient, and natural technique for bacteria's durability when exposed to environmental stress. Biofilm is a complex and completely natural structure that contains extracellular polysaccharide compounds, having a protective effect when faced with stress and extreme conditions. Hydrophobic polysaccharides restrict antibiotic entry and absorption into the network of biofilm and protect bacteria against the adverse effects of these antibacterial compounds (Aoudia et al., 2016; Grossova et al., 2017; Zhang et al., 2013). So, inspired by biofilm's protective structure against many environmental stresses, this study was designed to use this natural phenomenon to strengthen probiotic bacteria and increase their survival against antibiotics. Creating biofilm by bacteria is an innate strategy to maintain the bacterial community's survival against environmental stresses and antibiotic residues.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

#### 2.1.1 | Starter culture and inoculum preparation

Lyophilized culture of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* isolated from pickled cabbage was obtained from the Iranian Research Organization for Science and Technology. The microbial culture was activated according to the company's instructions. The activated bacteria were transferred into De Man, Rogosa, and Sharpe agar (MRS) (Oxoid, Milan, Italy) and incubated under anaerobic conditions at 37°C for 48–72 hr. The colonies were collected with a sterilized loop and suspended in sterile distilled water. The bacterial suspension was adjusted to ($10^9$ CFU/ml) by Spectrophotometers—UV Visible (Mecasys, Korea) to reach a target inoculum.

#### 2.1.2 | Preparation of antibiotics

Four widely used antibiotics were purchased from veterinary pharmacies, including tetracycline, oxytetracycline, sulfadimidine, and enrofloxacin. Several concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1,024 μg/ml) were prepared according to the active ingredient of each antibiotic (Zhang et al., 2013).

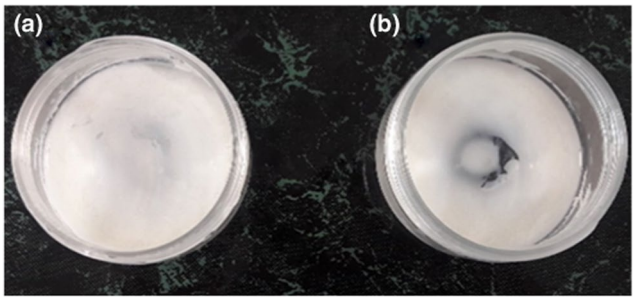


FIGURE 1 Biofilm formation in a container, (a) *Lactobacillus rhamnosus* and (b) *Lactobacillus plantarum*

### 2.2 | Methods

#### 2.2.1 | Biofilm assay on polystyrene microplates

One milliliter of strain suspension ($1.5 \times 10^9$ CFU/ml) was inoculated with 9 ml of fresh MRS broth culture and dispensed per well in a 24 well microplate; then, it was incubated at 30°C for 48 hr. After incubation, the medium was removed from each well, and the plates were washed twice to remove planktonic cells attached to the biofilm (Figure 1; Aoudia et al., 2016).

### 2.3 | Determination of minimum inhibitory concentration of antibiotic (MIC)

One hundred eighty microliters of culture medium containing $1.5 \times 10^9$ CFU/ml from each strain were poured into each well. Then, 20 μl of each antibiotic concentration was added and incubated for 48 hr at 30°C. After incubation, the culture medium was drained from the wells and washed twice with 0.5 ml of 150 mM NaCl solution. The microplate was then stained for 45 min with 1 ml of 0.05% (vol/vol) of crystalline violet solution and washed twice. One milliliter of 96% ethanol (vol/vol) was added to each well, and the optical density was determined at 595 nm (Fricks-Lima et al., 2011).

### 2.4 | Biofilm formation in polystyrene containers

The biofilm formation method was developed and performing several experiments to produce biofilm in the milk environment. Eighteen milliliter of pasteurized fresh milk containing 3% fat were inoculated with 2 ml of strains suspension ($1.5 \times 10^9$ CFU/ml) in a polystyrene container, the container used in this study had a diameter of 65 mm, a height of 55 mm, a volume of 150 ml, and a tightly closed lid. Then, it was incubated for 48 hr at 30°C. Finally, it was kept at 4°C after washing (Aoudia et al., 2016).

## 2.5 | Determination of viability of probiotic microorganisms in biofilm

The viability of *Lactobacillus* strains in the biofilms was tested in the storage period (3 days) at 4°C. For each test, 1 g of the biofilm samples (1 ml) of each biofilm solution was mixed with 9 ml of sterile peptone water (1 g/L). After sequential dilutions, appropriate dilutions were plated on set MRS. Then, they were incubated in an anaerobic jar with C type gas pack sachet (Merck KGaA, Darmstadt, Germany) at 37°C for 72 hr. The total counts of the viable bacteria were reported as logarithmic colony forming units per gram (log CFU/g). All the experiments were performed in triplicate, which means that each experiment was repeated at least three times (Li et al., 2017).

## 2.6 | Evaluation of antibiotic susceptibility of probiotics in biofilm and planktonic forms in yogurt containing antibiotics

Fresh milk with 3% fat was heated at 92°C for 10 min. It was then cooled to 42°C, and a micro milk brand yogurt starter was added to it (3.6% wt/vol). The last concentration of each antibiotic that the probiotics could not grow in the previous step was prepared. It was then added to the milk and mixed thoroughly. 100 ml of antibiotic-contaminated milk was added to each container containing the biofilm (Figure 2(c)). Also, in planktonic samples, 1 ml of the solution with $1.5 \times 10^9$ CFU/ml of strains was added to 9 ml of milk (10 to 90 ml of antibiotic-contaminated milk; Figure 2(a)). The control sample was antibiotic-free yogurt containing biofilm (Figure 2(b)). All samples were incubated at 42°C till the pH reached 4.6 then stored at 4°C to be assessed (Li et al., 2017; Yangilar & Yildiz, 2018).

## 2.7 | Enumeration of probiotic cells in planktonic and biofilm form in yogurt

The evaluation of probiotic microorganisms was performed by the spread plate method. After biofilm formation, the viable probiotic bacteria were plated on MRS agar containing 10 mg/L of vancomycin using spread plate method at intervals of 1, 2and 3 days of incubation in triplicate and determined after incubating at 37°C for 72 hr.

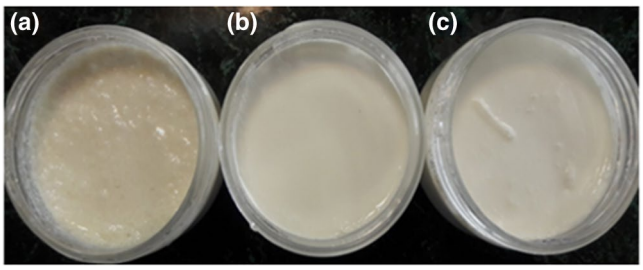


FIGURE 2 Antibiotic susceptibility test in yogurt produced with enrofloxacin contaminated milk: (a) planktonic form, (b) control: biofilm (antibiotics free), and (c) biofilm

The identification of *Lactobacills* strains was based on colony morphology (Li et al., 2017).

## 2.8 | Biofilms microstructure

Biofilm was fixed in 2.5% glutaraldehyde solution and 10 mM sodium cacodylate buffer for 4 hr at 4°C. It was then washed three times for 15 min in the sodium cacodylate sodium buffer with gentle mixing at room temperature. Subsequently, it was dehydrated in a graded ethanol series (50%, 70%, 80%, 90%, 95%, and 100%). The samples were placed on a special stub of the *SEM* device and then air-dried. Next, they were coated with Au-Pb (gold-palladium) for 18 s using the SC7620 Sputter Coater (UK). Afterward, it was examined by *SEM* device model LEO1450Vp made in Germany with a resolution of 2.5 nm and a maximum voltage of 35 kW. Images were taken at 20 kW at various magnifications (Figures 3 and 4) (Kubota et al., 2008).

## 2.9 | Statistical analysis

The experiment was performed according to a completely randomized factorial design with three replications. Analysis of variance (ANOVA) was performed using Minitab software (Minitab Release 19, Minitab Inc., and the USA). The Tukey method was used at a 5% significance level to compare the significant differences in treatment means.

# 3 | RESULTS

## 3.1 | Determination of minimum inhibitory concentration of antibiotic (MIC)

The results showed that enrofloxacin acted as the strongest antibiotics on the growth of the bacteria and could limit the growth of *L. rhamnosus* at 256 μg/ml and the growth of *L. plantarum* at 16 μg/ml. Both bacteria grew very poorly in the presence of tetracycline and oxytetracycline antibiotics up to 256 μg/ml and in the presence of sulfadimidine up to 512 μg/ml (Table 1).

## 3.2 | Survival of probiotics in biofilm

As shown in Table 2, the results of this study show that the survival of both probiotic strains during 72 hr has been almost constant and has not experienced severe and significant fluctuations ($p$-value > .05).

## 3.3 | Evaluation of antibiotic susceptibility

The antibiotic susceptibility of biofilm and planktonic form of probiotics are given in Table 3, showing a significant protective effect

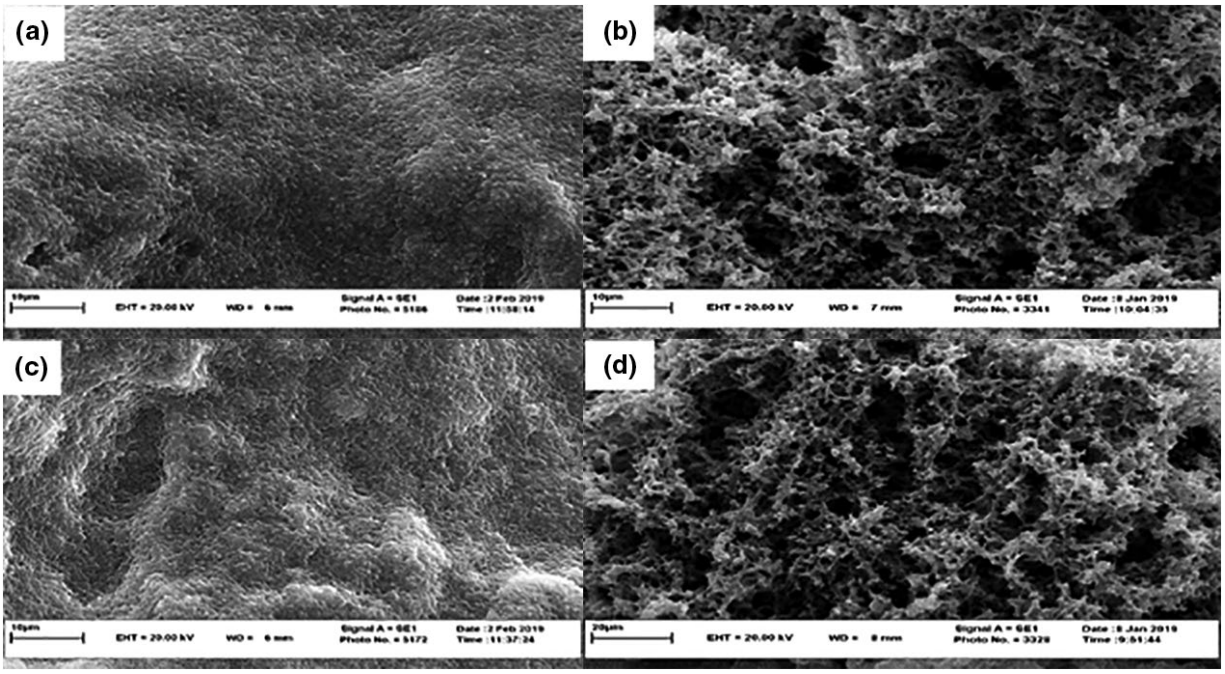


FIGURE 3 Scanning electron microscopy images of biofilm-forming *Lactobacillus rhamnosus* and *Lactobacillus plantarum* in MRS agar (a, c) and biofilm-forming *L. rhamnosus and L. plantarum* in milk (b, d)

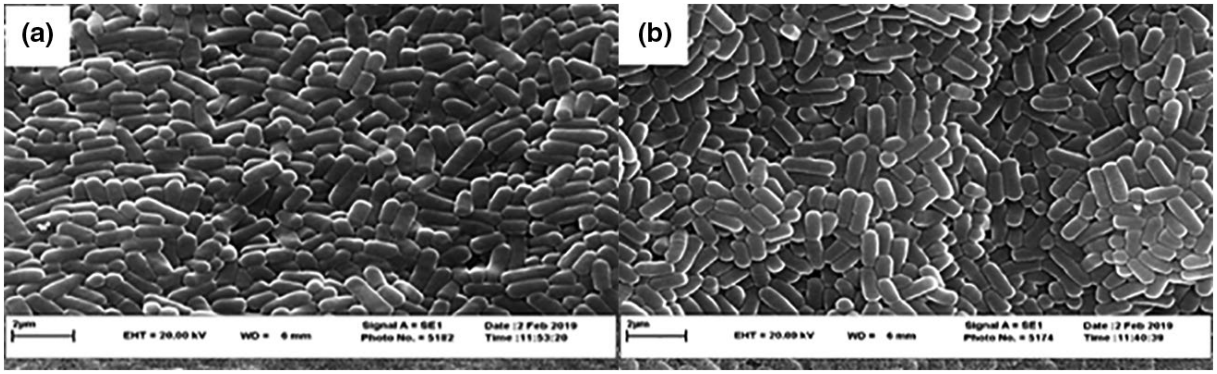


FIGURE 4 Scanning electron microscopy images of the planktonic cell of *Lactobacillus plantarum* (a) and *Lactobacillus rhamnosus* (b) in MRS agar

TABLE 1 The minimum inhibitory concentration of antibiotic (MIC) in vitro

| | Strain | |
|---|---|---|
| Antibiotics | *Lactobacillus rhamnosus* (μg/ μl) | *Lactobacillus plantarum* (μg/ μl) |
| Tetracycline | 256 | 256 |
| Oxytetracycline | 256 | 256 |
| Enrofloxacin | 256 | 16 |
| Sulfadimidine | 512 | 512 |

of biofilm on the survival of probiotic bacteria against antibiotics ($p_{value} \leq .05$). Also, antibiotic type has a significant effect on the probiotic survival rate ($p_{value} \leq .05$). On the other hand, the protective behavior of biofilm against different types of antibiotics has varied.

TABLE 2 Probiotic viability in biofilm (log CFU/ml)

| | Time (day) | | | |
|---|---|---|---|---|
| Treatment | 0 | 1 | 2 | 3 |
| *Lactobacillus rhamnosus* | 8.5 ± 0.6 | 8.7 ± 0.7 | 8.9 ± 0.8 | 8.8 ± 0.1 |
| *Lactobacillus plantarum* | 8.4 ± 0.6 | 8.6 ± 0.1 | 8.9 ± 0.3 | 8.7 ± 0.08 |

Furthermore, enrofloxacin was the most effective antibiotic in the survival reduction of probiotics in biofilm and planktonic forms, while the antibiotic sulfadimidine was the least effective antibiotic.

The results showed that yogurt produced with biofilm was not significantly different ($p$-value > .05) from the control sample in terms of consistency and even clotting and closing time. However,

the comparison of planktonic samples with control samples concerning yogurt consistency showed a significant difference ($p$-value < .05). The consistency of yogurt contaminated with antibiotics was lower than the control sample. Also, because of antibiotics, the closing time of yogurt and clot formation was longer than the control sample (Table 3).

### 3.4 | Biofilms microstructure

Images of biofilm structure with electron microscopy (Figure 3) compared to planktonic form (Figure 4) indicate a cohesive, three-dimensional structure of the biofilm that, as a strong skeleton, has been able to maintain this unique biological network. Probiotic bacteria in this cohesive and natural structure have gained more resistance than their counterparts in the planktonic state against environmental stress such as temperature changes, pH, and the presence of antibiotic residues, so their survival rate has increased.

## 4 | DISCUSSION

Field research has shown that oxytetracycline and enrofloxacin have long been used as strong therapeutic drugs for a wide range of animal diseases. Therefore, arbitrary administration of these drugs has been very common among traditional ranchers. Numerous research results indicate the presence of antibiotic residues in dairy products. Therefore, antibiotic residues should be considered a serious problem in the health and food field (Ma & Zhai, 2014; Pan & Chu, 2016; Rana et al., 2019). Previous studies confirm that a few numbers of probiotic species have been resistant to tetracycline. An important step in distinguishing intrinsic and acquired antibiotic resistance in probiotic bacteria is determining and comparing antibiotic susceptibility patterns of different strains. Although efforts have been made to do so, work has been done only on certain antibiotics and specific strains of *Lactobacillus* (Gueimonde et al., 2013). Chang Liu et al demonstrated that among 13 *Lactobacillus* species that were studied, none of them were resistant to tetracycline or were highly sensitive (Liu et al., 2009). In the present study, investigating antibiotic resistance of probiotic strains in the laboratory showed that the two studied strains (*L. rhamnosus* and *L. plantarum*) in planktonic form against antibiotics, which are widely used in veterinary, especially enrofloxacin, tetracycline, and oxytetracycline, were sensitive. Therefore, due to the nutritional and health importance of these beneficial bacteria (Hossain et al., 2017), the issue of their survival against adverse environmental factors, especially the host digestive system, stresses during the production of probiotic products. Most importantly, antibiotic residues are of great significance (Gueimonde et al., 2013; Plessas et al., 2012; Terpou et al., 2017). The solution examined in this study was to use the biofilm production technique by the bacteria to increase the survival of probiotics. Since few studies have been carried out on the effects of environmental and nutritional conditions on biofilm formation and simultaneously, the effect of antibiotics has rarely been investigated, the effect of biofilm formed in milk on probiotic resistance to the spectrum of antibiotics was investigated in this study for the first time. The results showed that both *L. plantarum* and *L. rhamnosus* strains have good biofilm production power. The biofilm produced by both strains was maintained for 72 hr, and probiotic viability was assessed. This result seems very desirable considering the industrial application of biofilm because biofilm survival indicates the biofilm's power to protect and maintain balance in the bacterial community. On the other hand, since time is important in the industry, Providing a technique is useful to the industry when it is not time-consuming and costly, so biofilm with a high ability to maintain and viability of probiotics can be effective in produce and with mass production of biofilm, it is possible to save time in producing a new product (Grossova et al., 2017). The biofilm's three-dimensional structure can also act as a strong biological substrate and provide the nutritional needs of bacteria for a long time and increase survival (Salas-Jara et al., 2016). As shown in Figure 3, the various channels created by the water in the biofilm network play an important role in meeting bacteria's nutritional needs. Extracellular polysaccharide compounds (EPS) help strengthen bacterial bonds and protect them from environmental pressures against the environment (Dertli et al., 2015; Salas-Jara et al., 2016). Also, the antibiotic susceptibility evaluation of probiotics in the biofilm form in the yogurt environment compared to their planktonic form showed an increase in biofilm bacteria's survival rate. So, using the biofilm technique, the survival rate of probiotic bacteria can be increased in higher concentrations of antibiotics. Furthermore, another concern about antibiotics is developing antibiotic resistance and its transmission through bacteria (He et al., 2019). Therefore, it can be pointed

**TABLE 3** Biofilm and planktonic antibiotic susceptibility (log CFU/ml)

| | | Antibiotics | | | |
|---|---|---|---|---|---|
| **Strain** | **Control** | **Sulphadimidin** | **Oxytetracycline** | **Tetracycline** | **Enrofloxacin** |
| Biofilm of *Lactobacillus rhamnosus* | $8.6 \pm 0.28^{a}$ | $8.6 \pm 0.28^{a}$ | $7.4 \pm 0.15^{b}$ | $6.11 \pm 0.16^{c}$ | $6 \pm 0.1^{c}$ |
| Planktonic form *L. rhamnosus* | $7.65 \pm 0.49^{a}$ | $6.8 \pm 0.76^{a}$ | Nd* | Nd* | Nd* |
| Biofilm of *Lactobacillus plantarum* | $7.5 \pm 0.14^{a}$ | $7.4 \pm 0.8^{a}$ | $6.1 \pm 0.3^{c}$ | $6.8 \pm 0.5^{c}$ | $6.05 \pm 0.2^{c}$ |
| Planktonic form *L. plantarum* | $7.5 \pm 0.14^{a}$ | $6.5 \pm 0.5^{a}$ | Nd* | Nd* | Nd* |

*Note:* Index letters indicate the comparison of the averages in the columns ($p$ value ≤ .05).
*Nd, not detectable.

out that since the biofilm creates a hydrophobic structure, it prevents the penetration of antibiotics and any antibacterial substances into the internal network and does not allow the entry of any antibiotics, which can be one of the most important desirable achievements of this technique (Salas-Jara et al., 2016).

Comparing the samples of yogurt produced in the form of biofilm with the control sample did not show a significant difference ($p$-value > .05), which could be an apparent reason for the function and valuable properties of the biofilm structure that can preserve the bacterial population (Salas-Jara et al., 2016; Zhang et al., 2013). However, in planktonic form, the samples were significantly different ($p$-value < .05) from the control sample in terms of yogurt consistency and the amount of synergy, which can be concluded that these defects in the product are due to the disturbance of the fermentation process and the loss of essential yogurt bacteria and probiotics due to the presence of antibiotics. Therefore, it refers to the protective effect of biofilm on the viability of bacteria (Grossova et al., 2017; Salas-Jara et al., 2016). Another industrial achievement of this study is that, in the dairy industry, stabilizers such as pectin and gum are used to reduce or prevent syneresis or the protein content is increased. In the present study, consistency was high in the yogurt samples containing biofilm compared to the planktonic form due to the biofilm's three-dimensional structure. Leccese Terraf et al. (2016) evaluated the biofilm matrix formed by *L. rhamnosus* CRL 1,332, demonstrating that the biofilm matrix contains large amounts of polysaccharides, carbohydrates, and proteins (Leccese Terraf et al., 2016). These natural compounds produced by probiotic bacteria in the biofilm network can play the same role as industrial stabilizers. Due to their hydrophilic groups, they can absorb yogurt water and reduce industrial stabilizers' consumption.

## 5 | CONCLUSION

In this study, biofilm, a unique method, was studied to develop novel generation of probiotics. Incorporating probiotic biofilm into yogurt increases the viability of probiotic strains and can affect the product's physical and mechanical properties. The probiotic biofilm exhibited the best rank in protecting the *L. plantarum* and *L. ramnosus*. This technique can significantly affect the survival of probiotics compared to similar methods such as encapsulation, microencapsulation, nanocomposite, and trapping. Therefore, considering this method's introduction as a new and innovative approach, it can protect probiotics against environmental stress and antibiotic residues. Moreover, due to the economic efficiency and lack of modern technologies, this method can be easily developed in the food and pharmaceutical industries.

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## AUTHOR CONTRIBUTIONS

**Zeinab Rezaei:** Investigation; Methodology; Software; Writing-original draft; Writing-review & editing. **Saeid Khanzadi:** Conceptualization; Methodology; Supervision; Writing-review & editing. **Amir Salari:** Conceptualization; Funding acquisition; Methodology; Project administration; Software; Supervision; Validation; Writing-review & editing.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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## REFERENCES

Afzaal, M., Saeed, F., Arshad, M. U., Nadeem, M. T., Saeed, M., & Tufail, T. (2019). The effect of encapsulation on the stability of probiotic bacteria in ice cream and simulated gastrointestinal conditions. *Probiotics and Antimicrobial Proteins*, *11*(4), 1348–1354. https://doi.org/10.1007/s12602-018-9485-9

Aoudia, N., Rieu, A., Briandet, R., Deschamps, J., Chluba, J., Jego, G., Garrido, C., & Guzzo, J. (2016). Biofilms of *Lactobacillus plantarum* and *Lactobacillus fermentum*: Effect on stress responses, antagonistic effects on pathogen growth and immunomodulatory properties. *Food Microbiology*, *53*, 51–59. https://doi.org/10.1016/j.fm.2015.04.009

Ashraf, R., & Shah, N. P. (2011). Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. *International Food Research Journal*, *18*(3), 837–853.

Bacanlı, M., & Başaran, N. (2019). Importance of antibiotic residues in animal food. *Food and Chemical Toxicology*, *125*, 462–466. https://doi.org/10.1016/j.fct.2019.01.033

Baeza, A., Urraca, J., Chamorro, R., Orellana, G., Castellari, M., & Moreno-Bondi, M. (2016). Multiresidue analysis of cephalosporin antibiotics in bovine milk based on molecularly imprinted polymer extraction followed by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, *1474*, 121–129. https://doi.org/10.1016/j.chroma.2016.10.069

Comunian, R., Paba, A., Dupré, I., Daga, E., & Scintu, M. (2010). Evaluation of a microbiological indicator test for antibiotic detection in ewe and goat milk. *Journal of Dairy Science*, *93*(12), 5644–5650. https://doi.org/10.3168/jds.2010-3474

Dertli, E., Mayer, M. J., & Narbad, A. (2015). Impact of the exopolysaccharide layer on biofilms, adhesion and resistance to stress in *Lactobacillus johnsonii* FI9785. *BMC Microbiology*, *15*(1), https://doi.org/10.1186/s12866-015-0347-2

FAO. (2016). Food and agriculture organization of the United Nations. Officer, P. FAO, Italy.

Fricks-Lima, J., Hendrickson, C., Allgaier, M., Zhuo, H., Wiener-Kronish, J., Lynch, S., & Yang, K. (2011). Differences in biofilm formation and

antimicrobial resistance of *Pseudomonas aeruginosa* isolated from airways of mechanically ventilated patients and cystic fibrosis patients. *International Journal of Antimicrobial Agents*, *37*(4), 309–315. https://doi.org/10.1016/j.ijantimicag.2010.12.017

Grossova, M., Rysavka, P., & Marova, I. (2017). Probiotic biofilm on carrier surface: A novel promising application for food industry. *Acta Alimentaria*, *46*(4), 439–448. https://doi.org/10.1556/066.2017.46.4.6

Gueimonde, M., Sánchez, B., de los Reyes-Gavilán, C. G., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology*, *4*, https://doi.org/10.3389/fmicb.2013.00202

Gul, O. (2017). Microencapsulation of *Lactobacillus casei* Shirota by spray drying using different combinations of wall materials and application for probiotic dairy dessert. *Journal of Food Processing and Preservation*, *41*(5), e13198. https://doi.org/10.1111/jfpp.13198

He, T., Long, S., Mahfuz, S., Wu, D., Wang, X., Wei, X., & Piao, X. (2019). Effects of probiotics as antibiotics substitutes on growth performance, serum biochemical parameters, intestinal morphology, and barrier function of broilers. *Animals*, *9*(11), 985. https://doi.org/10.3390/ani9110985

Hossain, M. I., Sadekuzzaman, M., & Ha, S. D. (2017). Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. *Food Research International*, *100*, 63–73. https://doi.org/10.1016/j.foodres.2017.07.077

Kellnerová, E., Navrátilová, P., & Borkovcová, I. (2014). Effect of pasteurization on the residues of tetracyclines in milk. *Acta Veterinaria Brno*, *83*(10), S21–S26. https://doi.org/10.2754/avb201483s10s21

Kjeldgaard, J., Cohn, M. T., Casey, P. T., Hill, C., & Ingmer, H. (2012). Residual antibiotics disrupt meat fermentation and increase risk of infection. *mBio*, *3*(5). https://doi.org/10.1128/mbio.00190-12

Kubota, H., Senda, S., Nomura, N., Tokuda, H., & Uchiyama, H. (2008). Biofilm formation by lactic acid bacteria and resistance to environmental stress. *Journal of Bioscience and Bioengineering*, *106*(4), 381–386. https://doi.org/10.1263/jbb.106.381

Leccese Terraf, M. C., Juárez Tomás, M. S., Rault, L., Le Loir, Y., Even, S., & Nader-Macías, M. E. F. (2016). Biofilms of vaginal *Lactobacillus reuteri* CRL 1324 and *Lactobacillus rhamnosus* CRL 1332: Kinetics of formation and matrix characterization. *Archives of Microbiology*, *198*(7), 689–700. https://doi.org/10.1007/s00203-016-1225-5

Li, C., Song, J., Kwok, L.-Y., Wang, J., Dong, Y., Yu, H., Hou, Q., Zhang, H., & Chen, Y. (2017). Influence of *Lactobacillus plantarum* on yogurt fermentation properties and subsequent changes during postfermentation storage. *Journal of Dairy Science*, *100*(4), 2512–2525. https://doi.org/10.3168/jds.2016-11864

Liu, C., Zhang, Z. Y., Dong, K., Yuan, J. P., & Guo, X. K. (2009). Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. *Biomedical and Environmental Sciences*, *22*(5), 401–412. https://doi.org/10.1016/s0895-3988(10)60018-9

Ma, J., & Zhai, G. (2014). Antibiotic contamination: A global environment issue. *Journal of Bioremediation & Biodegradation*, *5*, 5–6. https://doi.org/10.4172/2155-6199.1000e157

Mahendradatta, M., Bastian, F., & Amaliah, N. (2007). Shelf-life prediction of seasoning powder made from whole fermented fish (peda) by using arrhenius method. IPB (Bogor Agricultural University), 221–233.

Moghadam, M. M. (2016). Evaluation of antibiotic residues in pasteurized and raw milk distributed in the South of Khorasan-e Razavi province, Iran. *Journal of Clinical and Diagnostic Research*, https://doi.org/10.7860/jcdr/2016/21034.9034

Mohan, A., Hadi, J., Gutierrez-Maddox, N., Li, Y., Leung, I. K. H., Gao, Y., Shu, Q., & Quek, S. Y. (2020). Sensory, microbiological and physicochemical characterisation of functional manuka honey yogurts containing probiotic *Lactobacillus reuteri* DPC16. *Foods*, *9*(1), 106. https://doi.org/10.3390/foods9010106

Movassagh, M. H., & Karami, A. R. (2011). Beta-lactam antibiotics residues in pasteurized milk by beta star test in the North West region of Iran. *ARPN Journal of Agricultural and Biological Science*, *6*(11), 7–10.

Nguyen, F., Starosta, A. L., Arenz, S., Sohmen, D., Dönhöfer, A., & Wilson, D. N. (2014). Tetracycline antibiotics and resistance mechanisms. *Biological Chemistry*, *395*(5), 559–575. https://doi.org/10.1515/hsz-2013-0292

Pan, M., & Chu, L. M. (2016). Adsorption and degradation of five selected antibiotics in agricultural soil. *Science of The Total Environment*, *545–546*, 48–56. https://doi.org/10.1016/j.scitotenv.2015.12.040

Plessas, S., Bosnea, L., Alexopoulos, A., & Bezirtzoglou, E. (2012). Potential effects of probiotics in cheese and yogurt production: A review. *Engineering in Life Sciences*, *12*(4), 433–440. https://doi.org/10.1002/elsc.201100122

Rahman, M. S., Hassan, M. M., & Chowdhury, S. (2021). Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh. *Heliyon*, *7*(8), e07739. https://doi.org/10.1016/j.heliyon.2021.e07739

Rana, M. S., Lee, S. Y., Kang, H. J., & Hur, S. J. (2019). Reducing veterinary drug residues in animal products: A review. *Food Science of Animal Resources*, *39*(5), 687–703. https://doi.org/10.5851/kosfa.2019.e65

Rowles, H. L. (2017). How are probiotics affected by antibiotics? *Annals of Clinical and Laboratory Research*, *5*(2), https://doi.org/10.21767/2386-5180.1000163

Salas-Jara, M., Ilabaca, A., Vega, M., & García, A. (2016). Biofilm forming *Lactobacillus*: New challenges for the development of probiotics. *Microorganisms*, *4*(3), 35. https://doi.org/10.3390/microorganisms4030035

Shori, A. B., Aboulfazli, F., & Baba, A. S. (2018). Viability of probiotics in dairy products: A review focusing on yogurt, ice cream, and cheese. In *Advances in biotechnology* (Chapter 6). Open Access eBooks.

Singh, P., Magalhães, S., Alves, L., Antunes, F., Miguel, M., Lindman, B., & Medronho, B. (2019). Cellulose-based edible films for probiotic entrapment. *Food Hydrocolloids*, *88*, 68–74. https://doi.org/10.1016/j.foodhyd.2018.08.057

Terpou, A., Bekatorou, A., Kanellaki, M., Koutinas, A. A., & Nigam, P. (2017). Enhanced probiotic viability and aromatic profile of yogurts produced using wheat bran (*Triticum aestivum*) as cell immobilization carrier. *Process Biochemistry*, *55*, 1–10. https://doi.org/10.1016/j.procbio.2017.01.013

Yangilar, F., & Yildiz, P. O. (2018). Effects of using combined essential oils on quality parameters of bio-yogurt. *Journal of Food Processing and Preservation*, *42*(1), e13332. https://doi.org/10.1111/jfpp.13332

Zhang, H., Xie, L., Zhang, W., Zhou, W., Su, J., & Liu, J. (2013). The association of biofilm formation with antibiotic resistance in lactic acid bacteria from fermented foods. *Journal of Food Safety*, *33*(2), 114–120. https://doi.org/10.1111/jfs.12030

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