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# COMPARISON OF ANTIFUNGAL PROPERTIES OF *LACTOBACILLUS RHAMNOSUS* AND *LACTOBACILLUS REUTERI* WITH POTASSIUM SORBATE IN THE IRANIAN UF-FETA CHEESE

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**ABSTRACT**

This study was aimed to compare antifungal properties of two lactobacilli strains with potassium sorbate in cheese. In this regard, the effect of 10-fold cell-free extract of *Lactobacillus rhamnosus* PTCC 1637 *and Lactobacillus reuteri* PTCC 1655 was studied on the growth of mold and yeast in the Iranian UF-Feta Cheese during a 60-day ripening period at 4 °C. Additionally, the antifungal effect of potassium sorbate (300 and 500 ppm) and mixture of potassium sorbate and cell-free extract of tested bacteria were evaluated. The results showed that yeast and mold growth was occurred in both control and treated chesses, however, after 30 days storage, mold and yeast count was significantly lower in the cell free supernatant-treated UF-Feta cheese in comparison to the control cheese. Addition of potassium sorbate, as expected, significantly reduced mold and yeast growth in UF-Feta cheese during ripening period than control. On the other hand the result showed that mixture of probiotic extract and potassium sorbate had more efficiency in mold and yeast growth reduction than when the sorbate potassium was used alone. The highest fungal growth inhibition was achieved by application of 2 mL cell-free extract of *L. reuteri* CCTP 1655 along with 500 ppm potassium sorbate. Generally, it could be concluded that cell-free supernatant of tested bacteria has potential for use as bio preservatives in cheese and although studied probiotic extracts are not as effective as sorbate potassium in preventing fungi and yeast growth, they can be useful in reduction of yeast and mold growth.

**Keywords:** Antifungal, bio preservative, cheese, lactic acid bacteria, natural

## INTRODUCTION

Filamentous molds and yeasts commonly cause spoilage in different food products such as fermented milk products, cheese, bakery products and many other food products **(Ribes *et al.*, 2018; Schnürer & Magnusson, 2005)**. Fungal spoilage of food lead to food and economical loss in all of the world **(Cheong *et al.*, 2014)**. Although mold and yeast have been utilized to produce different dairy products, fungi are the major organism that spoil many processed dairy foods **(Cheong *et al.*, 2014; Fente-Sampayo *et al.*, 1995; Tropcheva *et al.*, 2014)**. Fungal contamination of cheese is an important issue in the dairy industry and it can significantly influence technological process, economic output and sensory properties. Furthermore, production of mycotoxins by mold could endanger consumer health **(Cheong *et al.*, 2014; Kure & Skaar, 2019; Plockova *et al.*, 2001)**.

Different fungi such as *Aspergillus* spp., *Candida* spp., *Cladosporium* spp., *Debaryomyces* spp., *Galactomyces* spp., *Geotrichum* spp., *Mucor* spp., *Penicillium* spp., *Trichoderma* spp. and *Yarrowia* spp., are the important fungi that participate in the occurrence of problems in cheese production and its spoilage. However, *Penicillium* spp. and *Aspergillus* spp. are the most frequently fungi that isolated from the spoiled cheese **(Kim *et al.*, 2017; Kure & Skaar, 2019).**

Therefore, the growth of mold on the cheese can cause food safety problem and remarkable economic loss. In this regard, to prevent or decrease fungal contamination of cheese, it is required to apply preventative and/or reduction strategies that inhibit or decrease the mold growth **(Kure & Skaar, 2019)**. Beside of preventive methods such as regular cleaning and disinfection of equipment, filtration of the air, high-pressure air, various methods of packaging, cold storage and using of preservatives have been developed to control growth of mold in cheeses **(Bagheripoor *et al.*, 2018; Heydari & Pessarakli, 2010; Juneja *et al.*, 2012; Khorshidian *et al.*, 2018; Kure & Skaar, 2019; Schnürer & Magnusson, 2005)**. The latter and utilizing of food-grade chemical preservatives such as acetic, lactic, propionic, sorbic and benzoic acids, have been applied as one of the most common methods to control fugal spoilage and extend the shelf-life of cheeses **(Jeong *et al.*, 2014; Khorshidian *et al.*, 2018; Schnürer & Magnusson, 2005; Silva & Lidon, 2016)**. However, consumer concerns regarding the health risk of chemical preservatives in addition to demand for free additives foods, have induced food researchers to investigate various natural food additives. Different natural antioxidants and antimicrobials can be obtained from animal, plant and microbial sources **(Cheong *et al.*, 2014; Khorshidian *et al.*, 2018; Tiwari *et al.*, 2009; Yousefi *et al.*, 2020)**. Nisin, pediocin, natamycin, lactocin, acidophilin, bulgaricin, helveticin, plantaricin, and reuterin are the antimicrobials that can be obtain from microorganisms especially lactic acid bacteria (LAB) **(Gyawali & Ibrahim, 2014; Juneja *et al.*, 2012; Lucera *et al.*, 2012; Tiwari *et al.*, 2009)**. LAB and probiotics which have a long history of use in foods, show different beneficial health effects **(Khanniri et al., 2018; Khorshidian et al., 2016; Mohammadi *et al.*, 2017; Yousefi et al., 2017)**. LAB as starter cultures in producing of various foods, especially fermented dairy products, produce compounds that have antioxidant and antimicrobial properties **(Gyawali & Ibrahim, 2014; Hossain *et al.*, 2017; Rakib *et al.*, 2017)**. It has been reported that organic acids, phenolic compounds, hydroxyl fatty acids, hydrogen peroxide, reuterin, and proteinaceous compounds which produced by LAB and probiotics might exert antifungal properties **(Dalié *et al.*, 2010)**. Since, on one hand consumers are concerned about using synthetic preservative and on the other hand there is a considerable interest in the use of natural preservatives, the aim of this study was to evaluate the antifungal properties of cell-free extract of LAB in Iranian UF-Feta cheese and to compare this antifungal properties with potassium sorbate.

## MATERIAL AND METHODS

### Starter and probiotic cultures

Lyophilized culture of *Lactobacillus rhamnosus* PTCC 1637 *and Lactobacillus reuteri* PTCC 1655 was obtained from the Iranian Research Organization for Science and Technology (IROST). In order to activate the microorganisms, both lactobacilli strains were cultivated under anaerobic conditions by a gas pack system in de Man, Rogosa and Sharpe (MRS) broth at 37°C for 48 h. Direct vat starter

(DVS) including *Streptococcus thermophillus* and *Lactococcus lactis ssp cremoris* and *lactic* was obtained from DI-PROX (Levallois, Paris, France) in order to produce Iranian UF-Feta cheese. Rennet was obtained from *Rhizomucor miehei* of DSM Food Specialties (Seclin, France).

**Preparation of cell-free supernatants**

Overnight culture of both lactobacilli strains was cultivated in MRS broth and put in the shaking incubator (200 rpm at 37°C for 24 h). Then, the supernatant containing cell-free extract was obtained by centrifuging at 10000 rpm for 10 min at 4°C. The obtained cell-free supernatant was passed through 0.22 µm microfilter. After that each cell-free supernatant (10 mL) was concentrated by vacuum rotary evaporator (60°C, 90 rpm $min^{-1}$ under vacuum) to reached a 10 fold concentrated supernatant (1 ml) and finally was kept at refrigerator (4°C) until usage **(Gerez *et al.*, 2009)**.

**Production of Iranian UF-Feta Cheese**

The bactofugated, ultra-filtered and pasteurized milk with 34% dry matter was supplied from the Iran Dairy Industry Inc., Pegah Co (Tehran, Iran). The obtained retentate was entered into starter tank in where 1% starter cultures were added until pH reached to 6.2. After that microbial rennet from *Rhizomucor miehei* (DSM Food Specialities, Seclin, France) was added to the retentate batches. Then it was instantly transferred into the container (200g) and moved to coagulation tunnel (35°C, 20 min) in where the retentate was converted to pre-cheese mixture. After addition of probiotic extract, potassium sorbate and mixture of cell-free supernatants of each LAB and potassium sorbate into the cheese container, the cheese samples were exposed to the air for half an hour in order to be contaminated with fungi spores. In the following step, parchment paper was put on the cheese and 3% dry salt was spared. Following the sealing process with aluminum, the cheese samples were kept at 26-28°C for 24 h. Finally, the cheese samples were kept at 4°C for ripening (60 days) and analysis were performed at 15-day interval.

**Antifungal effect of cell- free supernatants of LAB**

In order to evaluate antifungal properties of LABs, 1 and 2 mL of 10-fold cell-free extracts of *L. rhamnosus* PTCC: 1673 and *L. reuteri* PTCC: 1655 were added under sterile condition into the container containing 200 gram of retentate. In order to accelerate mold spoilage, the cheese samples were exposed to the air for about 30 min. Microbial analyses were performed after 15, 30, 45 and 60 days of storage of Iranian UF-Feta Cheese.

**Antifungal effect of potassium sorbate and it combination with LABs extract**

Potassium sorbate (0.03 and 0.05%) alone and in combination with 1 and 2 mL of 10-fold cell-free extracts of tested LAB were added under sterile condition into the container containing 200 g of retentate. Pre-cheese mixture were exposed to the air for about 30 min for accelerating mold spoilage. Finally the microbial analysis of the chees samples were carried out after 15, 30, 45 and 60 days of storage

**Microbiological analysis**

In order to evaluate microbial analysis, firstly, the cheese samples were prepared by adding 10 g of cheese to 90 mL of ringer solution to make 10-fold dilution. Mold and yeast count were determined by incubating 0.1 ml of diluted sample on sabouraud 4% dextrose agar with chloramphenicol at 25°C for 3 to 7 days **(ISIRI, 2007)**. The results were reported by colony forming unit per milliliter of sample.

**Statistical analysis**

The experiments were carried out in triplicate and the data analyzed using SPSS 21.0 software package program. Statistical analysis of data was performed by one-way analysis of variance and Tukey's test. Values of $P < 0.05$ were regarded as significant.

**RESULT AND DISCUSSION**

The effect of cell-free extract of tested LAB on mold and yeast growth in UF-Feta cheeses during 60 day storage is shown in Figure 1. According to the Figure 1 it can be understood that growth of mold and yeast carried out in both treated and untreated contaminated UF-Feta cheese during ripening. However, mold and yeast counts were lower in the cell-free extract-treated UF-Feta cheese in comparison to the control sample. As shown in Figure 1 there was no significant differences between treated-UF cheese and control in the first 15 days of ripening. However, after that, the cheese samples containing cell-free supernatants of *L. reuteri* CCTP 1655 and *L. rhamnosus* PTCC 1637 showed significantly less mold and yeast growth than control. In addition, as expected, potassium sorbate was highly effective in reducing the growth of mold and yeast in contaminated UF-Feta cheese during ripening period than control (Figure 2). In accordance with our study, the effect of potassium sorbate on microbial properties of Savak tuulum cheese was studied by **Demir *et al.* (2017)**. Their results revealed that potassium sorbate exerts inhibitory effect on *E. coli*, *staphylococcus aureus* and mold in vacuum packaged Savak tuulum cheese. Similarly **Ozdemir and Demirci (2006)** studied the effect of potassium sorbate (0.05 kg/100 kg) on microbiological properties of Kashar cheese. They found that potassium sorbate addition to Kashar cheese resulted in a decrease in coliform, yeast and mold counts. Furthermore, they indicated that dry potassium sorbate in comparison to fluid potassium sorbate led to lower mold counts. Additionally it has been reported that yeast and mold counts was decreased by addition of potassium sorbate to cheese **(Aly, 1996; Aworh & Egounlety, 1985)**. In addition, it has been claimed that yeasts and molds growth were thoroughly prevented by addition of potassium sorbate to Kashar cheese **(Kivanç, 1992)**. In accordance with other studies, the result of this study showed that potassium sorbate exert inhibitory effect on fungal growth in contaminated UF-Feta cheese during a 60-day ripening period at 4° C. Furthermore, microbiological properties of Turkish white cheese as consequence of addition of sorbic acid and potassium sorbate to the brine (300, 500 and 700 ppm) was evaluated by **Yilmaz and Kurdal (2008)**. They found that addition of sorbic acid and potassium sorbate resulted in a decrease in total counts of yeasts and moulds during 90-day ripening period at 4 °C **(Yilmaz & Kurdal, 2008)**. The exact antimicrobial mechanism of sorbic acid and potassium sorbat is not completely explained. However it seems that inhibition of various microbial growth does not occur thorough exact mechanism (**Yilmaz & Kurdal, 2008)**. It has been reported that transport of carbohydrates into yeast cell is inhibited by sorbic acid. In various bacteria sorbic acid uncouples oxidative phosphorylation and inhibits oxidative and fermentative assimilation **(Dorko *et al.*, 2014; Kivanc, 1990; Oztek, 1983)**.

The effect of *L. reuteri* CCTP 1655 and *L. rhamnosus* PTCC 1637 cell-free extracts along with potassium sorbate on mold and yeast growth is shown in Figure 3 and Figure 4, respectively. The result showed that mixture of cell-free extract and potassium sorbate had more efficiency in mold and yeast growth reduction than when the potassium sorbate was used alone. According to the Figure 3 and 4 it could be found that that mold and yeast growth prevention was 7.7 and 14% higher in the samples containing 1 and 2 mL of tested bacterial extract and 300 ppm potassium sorbate, respectively, than the sample with only 300 ppm potassium sorbate. Furthermore, prevention of fungal growth was 6.7 and 9.1% higher in the samples that contained 1 and 2 mL of tested bacterial extract and 500 ppm potassium sorbate, respectively, than only the potassium sorbate (500 ppm)-treated chees.

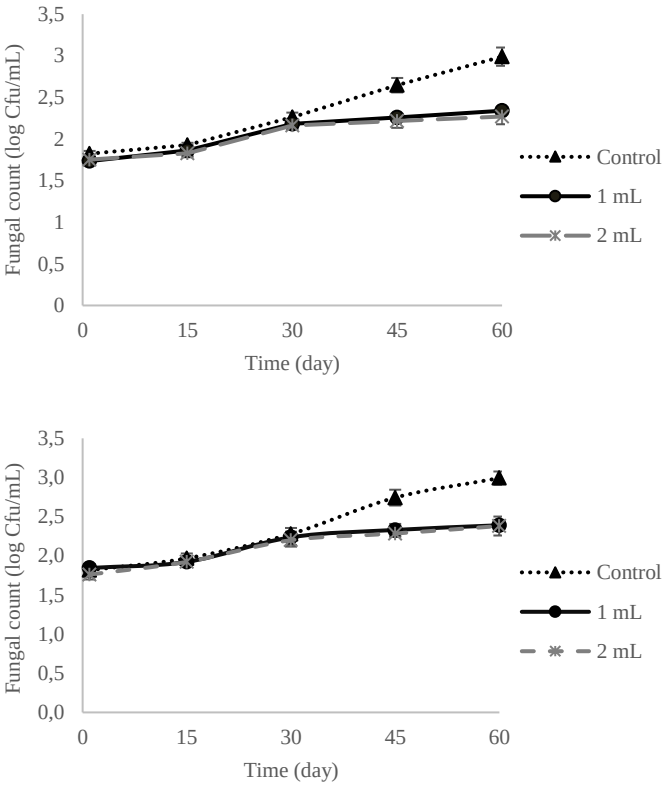


**Figure 1** The effect of cell-free extracts of lactic acid bacteria (a: *L. reuteri* CCTP 1655, b: *L. rhamnosus* PTCC 1637) on mold and yeast growth in UF-Feta cheese

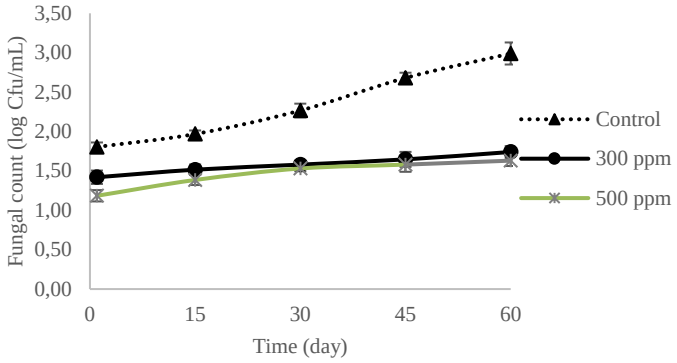


**Figure 2** The effect of potassium sorbate on mold and yeast growth UF-Feta cheese

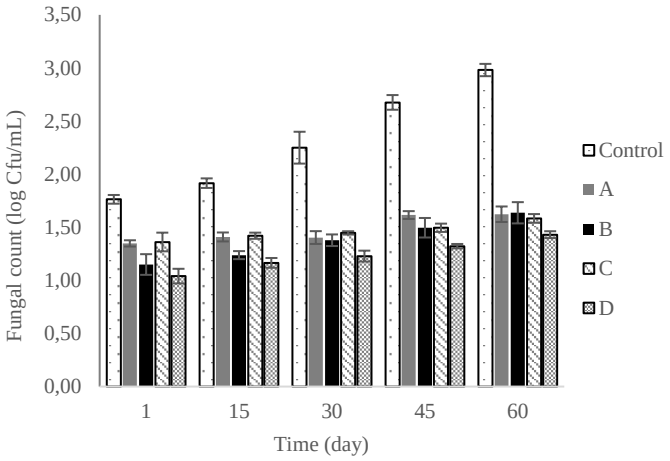


**Figure 3** Effect of potassium sorbate and cell-free extract of *L. reuteri* CCTP 1655 on mold and yeast growth in UF-Feta cheeses (A: *L. reuteri* CCTP 1655 (1 mL) + potassium sorbate (300 ppm), B: *L. reuteri* CCTP 1655 (1 mL) + potassium sorbate (500 ppm), C: *L. reuteri* CCTP 1655 (2 mL) + potassium sorbate (300 ppm), D: *L. reuteri* CCTP 1655 (2 mL) + potassium sorbate (500 ppm)

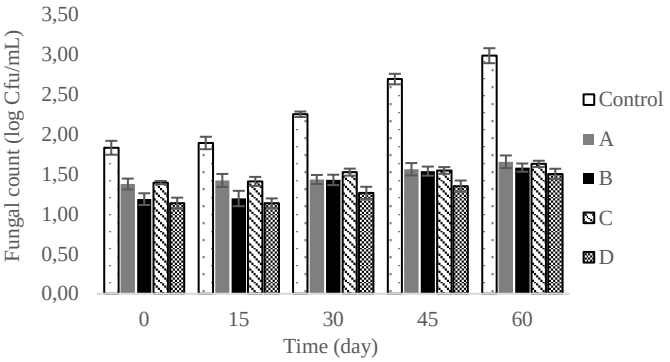


**Figure 4** Effect of potassium sorbate and cell-free extract of *L. rhamnosus* PTCC 1637 on mold and yeast growth in UF-Feta cheeses (A: *L. rhamnosus* PTCC 1637 (1 mL) + potassium sorbate (300 ppm), B: *L. rhamnosus* PTCC 1637 + potassium sorbate (500 ppm), C: *L. rhamnosus* PTCC 1637 (2 mL) + potassium sorbate (300 ppm), D: *L. rhamnosus* PTCC 1637 (2 mL) + potassium sorbate (500 ppm)

As mentioned before, mold contamination of cheeses as the major issues in the dairy industry can remarkably affect technological process, economic output and sensory properties and therefore various preservatives such as potassium sorbate is used for fungal reduction. Generally potassium sorbate as GRAS (Generally Recognized as Safe) is used as the most common preservative in cheese to prevent mold growth **(Neetoo, 2016)**. On the other hand there is a growing attention to utilize natural antimicrobial such as herbs, microorganism, fruit and vegetable and etc., in controlling mold growth **(Khorshidian *et al.*, 2018; Lucera *et al.*, 2012)**. The results of this investigation showed that cell-free extracts of tested LAB can be considered as bio preservatives and although studied cell-free extracts are not as effective as potassium sorbate in preventing mold and yeast growth, they can be useful in reduction of yeast and mold growth. As can be seen in Figure 1 and Figure 2, studied cell-free extracts can be effective in retarding yeast and mold growth. Various studies evaluate antifungal properties of LABs *in vitro* as well as in food products. However, there is no study using LABs and probiotic extract as bio preservatives in cheese. However, what is clear is the presence of compounds in probiotic extracts that have antifungal effects. The antimicrobial activity of isolated probiotic bacteria from cheese and fermented product was evaluated by **Ali *et al.* (2013)**. They tested antifungal activity of 14 probiotic isolates against *Rhizoctonia solani* and *Fusarium oxysporum*. Their results showed that nearly 20-52% of fungal growth was inhibited by all tested bacterial **(Ali *et al.*, 2013)**. They indicated anti-microbial properties of LABs and probiotics could be ascribed to the production of lactic acid and acetic acid and other low-molecularmass metabolites, and/or cyclic dipeptides **(Ali *et al.*, 2013; Lavermicocca *et al.*, 2000)**. Additionally, it has been stated that both polar and non-polar amino acids composition of the antifungal compounds that generated by *Lactobacillus* strains can form cross-linkages such as disulfide bridges with other compounds in the cell surface of the fungi. Formation of these linkages could disturb the permeability of fungal cell membranes and therefore, leakage of nutrients, protein, sugar and DNA carried out **(Gacem & Ould El Hadj-Khelil, 2016; Gomaa *et al.*, 2018)**. Therefore, as observed in this study, the antifungal activity of cell-free extract of tested *Lactobacillus* in the UF-Feta cheese might be attributed to the anti-fungal compounds that presented in the cell- free extract.

Furthermore, **Cheong *et al.* (2014)** studied the antifungal activities of different lactic acid bacteria (LAB) isolated from various herbs, vegetables and fruits. They found that out of 897 isolated LABs, 36, 11 and 12 isolates had weak, moderate and strong antifungal activities, respectively. Their results also showed that 12 isolated from a range of various sources that had the highest antifungal ability belongs to *Lactobacillus plantarum*. Moreover, these 12 isolated LAB showed antifungal activities against *Penicillium commune* in cottage cheese in comparison to control or the samples containing LAB without antifungal activity. They concluded that isolated LABs could potentially use as bio preservatives in cheese. In agreement with other studies, our results showed that LABs can be considered as bio preservatives. As is evident from the data presented in Table 1, cell- free supernatant of two tested LABs was able to prevent fungal growth and *L. reuteri* CCTP 1655 was more effective than *L. rhamnosus* PTCC 1637. This can be ascribed to the difference in the presence and content of various potential antifungal compounds in the supernatants of each tested bacteria. As can be seen in Table 1 the highest fungal growth inhibition was achieved by treatment containing cell-free supernatant of *L. reuteri* CCTP 1655 (2 mL) and potassium sorbate (500 ppm) during a 60-day ripening period. After that treatment with cell-free supernatant of *L. rhamnosus* PTCC 1637 and potassium sorbate (500 ppm) had the highest antifungal activity during 60-day ripening period. It can be concluded that by addition of cell-free supernatant of either *L. reuteri* CCTP or *L. rhamnosus* PTCC, potassium sorbate efficiency increased in fungal growth inhibition. In fact based on the obtained results (Table 1) it can be seen that mixture of 2 mL of 10-fold cell-free supernatant of each bacteria and 300 ppm potassium sorbet resulted in the same fungal growth when only 500 ppm potassium sorbate was used. Ability of *Lactobacillus rhamnosus* VT1 and *Lactobacillus reuteri* CCM 3625 was studied in controlling of mold growth on milk agar plates by **Plockova *et al.* (2001)**. They understood that active growing cell or cell-free supernatant of both lactobacilli strains had antifungal activity and the active growing cells excreted higher antifungal activities in comparison to antifungal metabolites that presented in cell-free supernatants. They indicated that antifungal activities of *Lactobacillus reuteri* CCM 3625 is come from mixture of lactic, acetic and succinic acid while the ability of *Lactobacillus rhamnosus* VT1 in inhibition of mold growth is probably due to lactic acid and some metabolites with antifungal properties **(Plockova *et al.*, 2001)**. Furthermore, **Nyanzi *et al.* (2014)** investigated anti-candida activity of methanol extract of 13 probiotic *Lactobacillus* strains and they recognized that the extracts exhibited fungistatic rather than fungicidal activity.

**Table 1** The effect of cell-free extract of LAB, sorbate potassium and mixture of cell-free extract of LAB and potassium sorbate on mold and yeast growth in UF-Feta cheeses

| Treatments | Time (day) | | | | |
|---|---|---|---|---|---|
| | 1 | 15 | 30 | 45 | 60 |
| Control | 1.82±0.099$^{aE}$ | 1.93±0.025$^{aD}$ | 2.26±0.007$^{aC}$ | 2.65±0.054$^{aB}$ | 2.990.037$^{aA}$ |
| *L. reuteri* CCTP 1655 (1 mL) | 1.73±0.044$^{aE}$ | 1.86±0.067$^{aD}$ | 2.18±0.055$^{bC}$ | 2.26±0.039$^{cdB}$ | 2.34±0.029$^{bA}$ |
| *L. reuteri* CCTP 1655 (2 mL) | 1.75±0.099$^{aE}$ | 1.83±0.098$^{aD}$ | 2.1±0.044$^{bC}$ | 2.21±0.080$^{dB}$ | 2.27±0.098$^{cA}$ |
| *L. rhamnosus* PTCC 1637 (1 mL) | 1.84±0.0477$^{aC}$ | 1.92±0.0571$^{aC}$ | 2.24±0.115$^{abB}$ | 2.33±0.075$^{bAB}$ | 2.39±0.062$^{bA}$ |
| *L. rhamnosus* PTCC 1637 (2 mL) | 1.76±0.064$^{aE}$ | 1.92±0.039$^{aD}$ | 2.2±0.060$^{abC}$ | 2.2±0.046$^{bcB}$ | 2.39±0.082$^{bA}$ |
| Potassium sorbate (300 ppm) | 1.42±0.088$^{bD}$ | 1.52±0.066$^{bcCD}$ | 1.57±0.051$^{cBC}$ | 1.65±0.095$^{eAB}$ | 1.74±0.072$^{dA}$ |
| Potassium sorbate (500 ppm) | 1.18±0.072$^{cD}$ | 1.38±0.066$^{cC}$ | 1.53±0.033$^{cB}$ | 1.58±0.076$^{fgAB}$ | 1.63±0.064$^{efgA}$ |
| *L. reuteri* CCTP 1655 (1 mL) + Potassium sorbate (300 ppm) | 1.348±0.029$^{bB}$ | 1.41±0.046b$^{cB}$ | 1.403±0.062$^{eB}$ | 1.61±0.036$^{efA}$ | 1.622±0.073$^{efgA}$ |
| *L. reuteri* CCTP 1655 (1 mL) + Potassium sorbate (500 ppm) | 1.149±0.097$^{cCD}$ | 1.237±0.037$^{dC}$ | 1.377±0.054$^{eBC}$ | 1.495±0.092$^{hAB}$ | 1.663±0.082$^{efA}$ |
| *L. reuteri* CCTP 1655 (2 mL) + Potassium sorbate (300 ppm) | 1.361±0.058$^{bD}$ | 1.420±0.024$^{bcC}$ | 1.447±0.015$^{deBC}$ | 1.4950±0.039$^{hB}$ | 1.583±0.041$^{gA}$ |
| *L. reuteri* CCTP 1655 (2 mL) + Potassium sorbate (500 ppm) | 1.040±0.069$^{cD}$ | 1.164±0.046$^{dC}$ | 1.228±0.051f$^{BC}$ | 1.321±0.020$^{iB}$ | 1.430±0.032$^{iA}$ |
| *L. rhamnosus* PTCC 1637 (1 mL) + Potassium sorbate (300 ppm) | 1.375±0.063$^{bC}$ | 1.419±0.081$^{bcC}$ | 1.431±0.056$^{eC}$ | 1.559±0.077$^{fghB}$ | 1.653±0.079$^{eA}$ |
| *L. rhamnosus* PTCC 1637 (1 mL) + Potassium sorbate (500 ppm) | 1.184±0.073$^{cC}$ | 1.129±0.086$^{dC}$ | 1.452±0.065$^{eB}$ | 1.535±0.057$^{ghA}$ | 1.575±0.051$^{gA}$ |
| *L. rhamnosus* PTCC 1637 (2 mL) + Potassium sorbate (300 ppm) | 1.391±0.020$^{bC}$ | 1.407±0.056b$^{cC}$ | 1.522±0.044$^{cdB}$ | 1.543±0.042$^{ghB}$ | 1.630±0.035$^{efgA}$ |
| *L. rhamnosus* PTCC 1637 (2 mL) + Potassium sorbate (500 ppm) | 1.135±0.068$^{cD}$ | 1.134±0.059$^{dD}$ | 1.262±0.077$^{fC}$ | 1.348±0.059$^{iB}$ | 1.500±0.065$^{hA}$ |

The values are expressed as mean ± standard deviation for triplicate samples.
Different small letters in each column show significant differences ($P < 0.05$)
Different capital letters in the same line show significant differences ($P < 0.05$).

Various studies have stated that different metabolites of lactic acid bacteria and probiotics such as cyclic peptides, reuterin, organic acid (acetic, propionic, lactic and 3-phenyllacetic acid), hydrogen peroxide can involve in antifungal properties of LABs and probiotics **(Crowley *et al.*, 2013; Sadiq *et al.*, 2019; Schnürer & Magnusson, 2005)**. Furthermore, it has been indicated that some miscellaneous antifungal compounds such as penolic antioxidants, diacetyl, short cyclic polylactates and benzeneacetic acid might participated in antifungal activities of LABs (**Sadiq *et al.*, 2019; Sellamani *et al.*, 2016; Wang *et al.*, 2012)**. It has been reported that secondary metabolites such as organic acids, bacteriocins and hydrogen peroxide participated in antifungal activities of *Lactobacillus rhamnosus* L60 and *Lactobacillus fermentum* L23 **(Gerbaldo *et al.*, 2012)**. Given the results of this study and the ability of two tested strains in prevention of fungal growth it seems that cell-free supernatants of both strains probably contains some metabolites that have antifungal properties.

## CONCLUSION

The antifungal activity of cell-free supernatants of *L. reuteri* CCTP and *L. rhamnosus* PTCC, potassium sorbate and mixture of cell-free supernatants of tested bacteria with potassium sorbate was studied in the Iranian UF-Feta cheese. The results showed that both bacterial extracts were able to some extend decrease the fungal growth compared to control sample. As expected in the potassium sorbate treated cheese, yeast and mold count significantly decreased. Furthermore, mixture of bacterial extract and potassium sorbate had more efficiency in mold and yeast growth reduction than when the sorbate potassium was used alone. It seems that antifungal activities of tested bacteria can be originated from the various compounds such as organic acids low-molecularmass metabolites, cyclic dipeptides and hydrogen peroxide that presented in the cell-free extract of each bacteria. In general, it can be concluded that use of cell-free supernatant of tested *Lactobacillus* may be considered as bio preservative and therefore it might be a promising approach for producing organic foods free from any chemical preservatives.

**Conflict of interest:** The authors declare there are no conflicts of interest.

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