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AVALIAÇÃO DE PROPRIEDADES ANTIOXIDANTES E PRÓ-OXIDANTES DE ESTIRPES DE LACTOBACILLUS E ENTEROCOCCES USANDO O MÉTODO DE ORAC E LUX-BIOSENSOR

ASSESSING ANTIOXIDANT AND PROOXIDANT PROPERTIES OF LACTOBACILLI AND ENTEROCOCCI STRAINS BY USING ORAC AND LUX-BIOSENSOR METHODS

ОЦЕНКА АНТИОКСИДАНТНЫХ И ПРООКСИДАНТНЫХ СВОЙСТВ ШТАММОВ ЛАКТОБАЦИЛЛ И ЭНТЕРОКОККОВ С ПОМОЩЬЮ МЕТОДОВ ORAC И LUX-БИОСЕНСОР

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RESUMO

Os lactobacilos são amplamente utilizados na medicina como bactérias probióticas. Os lactobacilos são considerados um dos tipos mais importantes de micróbios intestinais. Essas bactérias têm um efeito antioxidante e protetor de genes no sistema imunitário e nervoso de seus portadores. Mas algumas estirpes de enterococos podem ser patógenos e causar doenças como infecções do trato urinário, bacteremia, infecções no local da cirurgia, infecções na corrente sanguínea, diarréia. A necessidade de estudar as propriedades benéficas e prejudiciais das bactérias para os seres humanos determina a relevância do estudo. As propriedades antioxidantes e pró-oxidantes de 11 estirpes de Enterococcus e 7 metabólitos das estirpes de Lactobacillus foram analisadas utilizando uma análise de capacidade de absorção de radicais de oxigênio (ORAC) e um teste de biossensor lux. As bactérias foram incubadas no leite de vaca. Leite não fermentado foi usado como controle. O estudo mostrou que o leite fermentado com enterococos não apresentou diferenças significativas na capacidade antioxidante em comparação ao controle. Por outro lado, quase todos os lactobacilos aumentaram a capacidade antioxidante do leite coalhado em comparação com o leite não fermentado. Ou seja, os metabólitos das estirpes de Lactobacillus mostraram fortes propriedades antioxidantes, mesmo em baixas concentrações. Os metabólitos das estirpes de Enterococcus possuíam propriedades pró-oxidantes. Eles aumentaram a ação de outros pró-oxidantes, como paraquat, peróxido de hidrogênio, dioxidina, e mostraram um efeito sinérgico. O teste do biossensor lux, usado para avaliar o efeito de substâncias em células vivas com um metabolismo complexo, foi mais informativo do que a análise ORAC, que nos permitiu avaliar as propriedades antioxidantes e pró-oxidantes dos metabólitos de bactérias probióticas. O estudo revelou não apenas a influência do efeito direto da substância de teste na molécula alvo, mas também o efeito da ação indireta, interferindo em outros processos bioquímicos de uma célula viva, o que confirmou a necessidade de usar o teste de biossensor lux para trabalhos adicionais ao escolher estirpes de bactérias probióticas.

Palavras-chave: *Lactobacillus, Enterococcus, produto de leite fermentado, antioxidante, pró-oxidante.*

ABSTRACT

Lactobacilli are widely used in medicine as probiotic bacteria. Lactobacilli are considered one of the

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most important types of intestinal microbes. These bacteria have an antioxidant, gene protective effect on the immune and nervous systems of the host. But some strains of enterococci can be pathogenic microorganisms and cause diseases such as urinary tract infections, bacteremia, infections at the surgical sites, bloodstream infections, diarrhea. The need to study the beneficial and harmful properties of bacteria for humans determines the relevance of the study. The antioxidant and prooxidant properties of 11 Enterococcus strains and 7 metabolites of Lactobacillus strains were analyzed using an oxygen radical absorption capacity (ORAC) analysis and a lux biosensor test. Bacteria were incubated in cow's milk. Unfermented milk was used as a control. A study showed that milk fermented with enterococci did not have significant differences in antioxidant ability compared to control. In contrast, almost all lactobacilli increased the antioxidant ability of sour milk compared to unfermented milk. That is, the metabolites of Lactobacillus strains have demonstrated strong antioxidant properties even at low concentrations. The metabolites of Enterococcus strains possessed prooxidant properties. They enhanced the action of other prooxidants, such as paraquat, hydrogen peroxide, dioxidine, and showed a synergistic effect. The Lux biosensor test, used to evaluate the effect of substances on living cells with a complex metabolism, was more informative than the ORAC analysis, which allowed to evaluate the antioxidant and prooxidant properties of probiotic bacteria metabolites. The study revealed not only the influence of the direct effect of the test substance on the target molecule, but also the effect of indirect action by interfering with other biochemical processes of a living cell, which confirmed the need to use the biosensor lux test for further work when choosing strains of probiotic bacteria.

Keywords: *Lactobacillus, Enterococcus, fermented milk, antioxidant, prooxidant.*

АННОТАЦИЯ

Бактерии р. Lactobacillus широко применяются в медицине в качестве пробиотических бактерий. Они, а также бактерии р. Enterococcus, составляют важную часть кишечной микробиоты, обладают антиоксидантной, генопротекторной активностью и влияют на иммунную и нервную систему хозяина. С другой стороны, некоторые представители р. Enterococcus могут оказаться патогенными и вызывать такие заболевания, как диарея, инфекции мочевыводящих путей, инфекции, развивающиеся при хирургических вмешательствах, в т.ч. инфекции кровотока. Именно поэтому важно изучить как положительные, так и негативные свойства данных бактерий. 11 штаммов энтерококков и 7 штаммов лактобацилл были исследованы на антиоксидантную и прооксидантную активность с помощью метода ORAC и Lux-биосенсорного теста. Бактрии инкубировали в коровьем молоке. В качестве контроля использовали неферментированное молоко. Молоко, ферментированное энтерококками, не имело существенных различий в антиоксидантной способности по сравнению с контролем. Напротив, почти все лактобациллы даже при низких концентрациях значительно увеличивали антиоксидантную активность ферментированного молока по сравнению с неферментированным. Метаболиты штаммов бактерий р. Enterococcus, напротив, обладали прооксидантными свойствами. Они показали синергетический эффект с действием других прооксидантов, таких как паракват, перекись водорода, диоксидин. Lux-биосенсорный тест, использованный для оценки действия метаболитов на живые клетки, оказался более информативным, чем метод ORAC. Он позволил оценить не только антиоксидантные, но и прооксидантные свойства метаболитов пробиотических бактерий. Lux-биосенсорный тест выявил не только прямое действие метаболитов на молекулу-мишень, но и опосредованное, происходящее путем вмешательства метаболитов в другие биохимические процессы живой клетки. Это подтверждает необходимость использования lux-биосенсорного теста при дальнейшей работе с пробиотическими штаммами.

Keywords: *Lactobacillus, Enterococcus, кисломолочный продукт, антиоксидант, прооксидант.*

1. INTRODUCTION

Lactobacilli are widely used in medicine and veterinary as probiotic bacteria (Acurcio *et al.,* 2014; Ruzicka *et al*., 2016; Alfaia *et al.,* 2018; Beatrice *et al.,* 2018; Maldonado *et al.,* 2018; Tarabees *et al*., 2019; Zhu *et al.,* 2019; Mekadim *et al.,* 2019; Petrut *et al.,* 2019; Barbieri *et al.,* 2019). The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Report of a joint…, 2006).

Lactobacilli are considered one of the most important types of intestinal microbes. Previous studies have proven that these bacteria have an antioxidant, genoprotective effect on the immune and nervous system of the host (Rybalchenko *et al*., 2014; Liévin-Le Moal and Servin, 2014; Saez-Lara *et al.,* 2015; Wang *et al.,*

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2016; Wang *et al,* 2017; Chistyakov *et al.,* 2018; Mekadim *et al*., 2018). In addition to lactobacilli, bifidobacteria are actively used as probiotics, and they have similar properties (Saez-Lara *et al.,* 2015; Wang *et al.,* 2016).

Probiotics affect the balance of intestinal microflora, suppressing enhanced inflammatory responses, immune system stimulation, prevent diarrhea instead of using antibacterial drugs (Bin, 1995; Burns and Rowland, 2000; de Roos and Katan, 2000; Marteau, 2001; Liévin-Le Moal and Servin, 2014; Saez-Lara *et al.,* 2015; Wang *et al.,* 2016). In addition to medications, often in everyday life people get probiotic bacteria and their metabolic products in their daily food. This is why cow milk was chosen as a culture medium for probiotic bacteria in this study. Milk itself has a rather high antioxidant activity (Khan *et al.,* 2017).

Currently, researchers are interested in studying enterococci, which are also main types of the intestinal microbiome (Dominguez-Bello *et al.,* 2010; Franz *et al.,* 2011). It is known that enterococci can also affect immune system regulation, normal intestinal microflora maintenance, antitumor activity, antimicrobial activity, antioxidant activity, and lowering cholesterol levels (Pieniz *et al.,* 2014; Molina *et al.,* 2015; Guo *et al.,* 2016; Li *et al.,* 2017). According to the literature, strains of all the presented lactobacilli species are able to exhibit a different spectrum of antioxidant activity (Corsetti *et al.,* 2008; Amaretti *et al.,* 2013; Mishra *et al.,* 2015).

On the other hand, some strains of enterococci can be pathogens and cause diseases such as urinary tract infections, bacteraemia, surgical site infections, bloodstream infections, diarrhea (Schaberg *et al.,* 1991; Foulquié Moreno *et al.,* 2006), and can be toxic in food production (Franz *et al.,* 2001). Disputes about the applicability of various strains of enterococci as probiotics are still ongoing (Ghosh and Zurek, 2015; Anadón *et al*., 2016; Joshi and Biswas, 2017; Carasi *et al.,* 2017; Li *et al.,* 2018; Igonina et al., 2018; Sparo et al., 2018; Popovic et al., 2018; Braïek *et al.,* 2019).

In this work, the antioxidant activity of lactobacilli and enterococci strains metabolites were investigated using two different methods. Oxygen Radical Absorbance Capacity (ORAC) assay, which is a common method for assessing the antioxidant activity of different substances and compounds, including bacterial metabolites (Mishra *et al.,* 2015); and the lux-biosensor test, a promising method to identify the interaction of substances occurring in vivo in a living cell (Manukhov *et al.,* 1999; Prazdnova *et al.,* 2015; Chistyakov *et al.,* 2018).

2. MATERIALS AND METHODS

2.1. Probiotic strains

The metabolites of 11 Enterococcus strains and 8 Lactobacillus strains were analyzed. 6 Enterococcus strains: *E. durans* 6380, *E. durans* 6363, *E. durans* 6379, *E. durans* 6413, *E. durans* 6451, *E. faecium* 9683, and 2 Lactobacillus strains: *L. rhamnosus* RH and *L. paracasei* 2647 were obtained from the collection of experimental mutagenesis laboratory. 5 Enterococcus strains: *E. durans* 61, *E. faecium* 67, *E. faecium* 75, *E. faecium* 81, *E. faecium* 115 and 6 Lactobacillus strains: *L. plantarum* 83, *L. acidophilus* 94, *L. casei* 100, *L. rhamnosus* 108, *L. casei* 116, *L. brevis* 122, were clinical isolates, kindly provided by Pokudina Inna Olegovna, laboratory "Biomedicine", SFedU. All strains were identified by MALDI-TOF mass spectrometry on a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with Biotyper software (version 3.0) (Bruker Daltonics).

All bacteria were cultured at 37°C in 10 ml cow milk for 3 days. Ultra-pasteurized cow milk brand "Prostokvashino", Danone-Unimilk, fat content of 2.5% was used. Supernatants were collected by centrifugation (Minispinplus; Eppendorf, Leipzig, Germany) of fermented milk at 6000 rpm for 7 min.

2.2. Lux-biosensor test

Escherichia coli strains MG1655 (pSoxS-lux) (obtained from Manukhov, State Scientific Center Genetika, Moscow, Russia) were used as Lux biosensors, identifying induction of Sox operon, which is involved in SOS-reparation and serve as a part of the cellular antioxidant defense system (Zavilgelsky *et al.,* 2007). Antioxidant activity was evaluated by the ability of bacterial metabolites to reduce Sox-response, stimulated by addition of dioxidine (2,3-Quinoxalinedimethanol,1,4-dioxide, Biosintez, Penza, Russia) up to 2.25×10^{-5} M, paraquat (N,N' -dimethyl-4,4' -bipyridinium dichloride, Sigma Aldrich, Saint-Louis, MO) up to 10^{-3} M and hydrogen peroxide (Ferrain) up to 10^{-3} M concentrations respectively (Prazdnova *et al.,* 2015). The obtained supernatants were consecutively diluted 10, 100, 1000 times with the above reagents. Milk and it's dilutions was used as a control.

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The methodology for Lux biosensors bioluminescence detection was thoroughly described by Manukhov *et al.* (1999). A brief description is provided below. Pre-incubation of supernatant with culture was performed for 30 min. For luminescence measurements, an LM-01A automatic microplate luminometer (Immunotech, Praha, Czech Republic) was used. Measurements were carried out every 10 min for 120 min. To evaluate the influence of studied factors on Sox operon expression, the induction factor (Is) was calculated according to Equation 1:

$$Is = (Le/Lk) - 1 \quad \text{(Eq. 1)}$$

where Lk and Le are luminescence intensities of control (without any substances, only lux-biosensors and water) and experimental samples (lux-biosensors with inducer or probiotic metabolites or both) respectively.

To characterize the protective activity of the studied concentration, the mean value of P during the whole duration of measurements was used. Each experiment was conducted at least three times in triplicate and the statistical analysis was performed using Student's t-test. Confidence intervals were calculated using MICROSOFT EXCEL (Microsoft Corporation, Redmond, WA) for P = 0.05.

2.3. ORAC assay

The total antioxidant capacity (TAC) was assessed using ORAC assay. It was carried out using an ORAC Assay Kit (ab233473) (Abcam plc., UK). The fluorescence intensity measurement was performed using a FLUOstar Omega, (BMG Labtech, Germany). The supernatants were diluted with fluorescein consecutively by 10, 100, 1000 times. Milk and its dilutions were used as a control (Amorati and Valgimigli, 2015; Mellado-Ortega *et al.*, 2017).

3. RESULTS AND DISCUSSION:

3.1. Determining antioxidant activity of microorganisms by the ORAC assay

The antioxidant capacity of studied microorganisms is shown in Table 1. Milk was used as a control, all bacteria were grown on milk. Milk fermented with enterococci had no significant differences in antioxidant capacity compared to control. In contrast, almost all lactobacilli increased the antioxidant capacity of fermented milk compared to not-fermented. There were no significant differences in ORAC value in the case of strains L. casei 100 and L. brevis 122, they did not significantly differ from non-fermented milk, while strains L. plantarum 83 and L. rhamnosus RH showed the most significant increase in antioxidant capacity compared to control by about two times. It should be noted that the activity of lactobacilli did not depend on the species.

3.2. Determining antioxidant activity of lactobacilli using lux-biosensors

Lactobacillus metabolites did not show prooxidant activity - the change in the luminescence induction factor of the biosensor did not significantly differ from the control (Table 2). The addition of an inducer (paraquat) caused a high increase in the luminescence of biosensors, (Is) paraquat ranged within 23.9-27.4. For estimations, an average value of 24.2 was used. Unfermented milk showed a high protective activity, reducing the luminescence level of the lux-biosensor by 26% at a volume in solution of 10% (Table 2). In less concentrated solutions, the protective activity of milk decreased to 9%. Almost all lactobacilli demonstrated varying antioxidant properties and protected the biosensor cells from the action of superoxide. The greatest protective effect showed by L. plantarum 83 (91%), L. rhamnosus RH (84%) and L. acidophilus 94 (70%). L. casei 100 and L. brevis 122 did not show significant results compared to the control.

The direct reaction of E. coli MG1655 PSoxS-lux with the lactobacilli and enterococci metabolites, and the ability of these metabolites to protect cells from the action of paraquat causes superoxide generation in the cell (Dinis-Oliveira *et al.,* 2008). Paraquat in reaction with enzymes of the respiratory chain of the cell leads to the superoxide generation (Dinis-Oliveira *et al.,* 2008). Hydrogen peroxide and dioxidine are also able to activate the SoxRS in vivo (Manchado *et al.,* 2000; Sycheva *et al.,* 2004). As the volume of supernatant in solution decreased, its effect also decreased. However, with a decrease in volume from 10% to 1%, the protective activity of the supernatant practically did not decrease. Even with a supernatant volume of 0.01%, the protective activity of fermented milk was still recorded, significantly exceeding the activity of unfermented milk (10-42%, depending on the strain).

3.3. Determining antioxidant activity of enterococci

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using lux-biosensors

The supernatant of Enterococcus fermented milk in a volume of 10% caused a significant increase in induction factor of the E. coli MG1655 PSoxS-lux (Table 3). E. durans 6380, E. durans 6413, E. faecium 9683 had the maximum prooxidant effect (3.7, 3.4, 3.2, respectively). A decrease in the supernatant content to 1% led to the disappearance of this effect. The simultaneous action of fermented milk and paraquat led to a significant increase in the induction factor. If (Is) of paraquat was 25.2, then adding 10% of supernatant and paraquat at the same time increased (Is) to 45.5 in the case of E. faecium 9683. The biggest increase was caused by E. faecium 9683, E. durans 6380, E. durans 6413, E. faecium 67 (an increase of 81%, 75%, 69% and 63%, respectively).

A decrease in the concentration of supernatant to 1% neutralized this effect in half of the studied strains, and a decrease to 0.1% neutralized this effect in all of these strains.When superoxide appears in the cell, the production of soxS gene products increases and the cell begins to glow brighter. Luminosity correlates with the amount of superoxide in the cell (Chistyakov *et al.,* 2018).

3.4. Studying the effect of enterococcal metabolites on the Sox response induced by various inducers

Hydrogen peroxide and dioxidine were used as inducers. The Enterococcus metabolites had a prooxidant effect. For comparison, (Is) of E. durans 6380 was - 3.5-3.7, (Is) of hydrogen peroxide at a concentration of 10-3 M – 5.4. The effect was observed only in high concentrations of the supernatant - 10%, sometimes 1% and quickly disappeared upon dilution. When superoxide inducer was introduced into the solution, Enterococcus metabolites led to a significant increase in the Sox response. Table 4 clearly showed that this is not a simple addition of the activity of metabolites and paraquat, but a synergistic effect. That is, the metabolites of Enterococcus do not have a strong prooxidant effect directly, but significantly increase the effect of paraquat. To find out if this effect is specific to paraquat, or it works similarly on other types of prooxidants, other inducers, such as hydrogen peroxide and dioxidine were used.

(Is) of the hydrogen peroxide was 5.4, of the dioxidine – 5.1. It is significantly lower than (Is) of paraquat. The fact is that E. coli MG1655 PSoxS-lux responds primarily to superoxide. Paraquat in reaction with enzymes of the respiratory chain of the cell leads to the superoxide generation. Hydrogen peroxide and dioxidine are also able to activate the SoxRS in vivo, therefore, they generated a similar response, but their induction factor was lower. However, enterococcal metabolites also showed a synergistic effect with these inducers, significantly increasing (Is) when combined.

Paraquat was used as a positive control, the obtained induction factors of paraquat slightly differ from those presented above, since this is a different experiment. In general, differences between different replicates are not significant. The metabolites of these strains significantly increased (Is) of dioxidine (up to 96% in the case of E. faecium 9683), and also increased (Is) of hydrogen peroxide (up to 52% in the case of E. durans 6451).

4. CONCLUSIONS:

Considering that milk itself has a rather high antioxidant activity, in all experiments the effect of metabolites of probiotic bacteria was studied by using unfermented milk as a control. The ORAC assay was used to evaluate antioxidant and prooxidant properties of probiotic bacteria metabolites. The metabolites of Enterococcus bacteria did not show any antioxidant activity compared to non-fermented milk. Lactobacillus bacteria varied greatly in their ability to synthesize substances with antioxidant properties.

The ability to form antioxidant metabolites in high concentrations is a feature of the strain rather than a species. Therefore, it seems promising to isolate the genetic characteristics of highly active strains in order to create a super-producer strain in the future. The same characteristics were evaluated by using another method, which allows to evaluate the effect of bacterial metabolites on the metabolism of living cells. Lux-biosensor E. coli MG1655 PSoxS-lux cells carrying plasmids with luxCDABE operon from the photobacterium Photorhabdus luminescens under the control of E. coli promoters. The production of soxS gene products increases and the cell begins to glow.

The direct reaction of E. coli MG1655 PSoxS-lux with the lactobacilli and enterococci metabolites, and the ability of these metabolites to protect cells were evaluated from the action of paraquat. Lactobacillus metabolites are powerful antioxidants significantly protect cells from the action of superoxide radical formed by paraquat.

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Moreover, they themselves do not negatively affect the Sox-response of the cell. Even in small concentrations (0.01% supernatant), a significant protective effect was observed. The strains that showed the highest antioxidant capacity when using the ORAC assay showed the most significant protective effect in the bioluminescent test. This suggests the possibility of comparing the results of studies obtained in these different methods.

Enterococcal metabolites also showed a synergistic effect with these inducers, significantly increasing (Is) when combined which means that these metabolites do not affect the stage of interaction of paraquat with the enzymes of the respiratory chain, but the stage of cell response to the appearance of free radicals. This effect should be considered when choosing enterococcal strains to create probiotic preparations. After all, the body is constantly exposed to prooxidants obtained from food and air, as well as produced in the cells of the body, and an increase in the number of intestinal enterococcus can increase the oxidative load on the body.

The lux-biosensor test was more informative than ORAC. It evaluates the effects of substances on living cells with a complex metabolism. Therefore, the study can reveal not only the effects of the direct action of test substance on the target molecule, but also the effects of the indirect action through intervention in other biochemical processes of the living cell. Therefore, it's recommended to use the lux-biosensor test while selecting probiotic bacteria strains for further work.

5. ACKNOWLEDGMENTS:

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Table 1. *Oxygen radical antioxidant capacity (ORAC) values of supernatants of fermented milk*

Strain	**ORAC value (μmol TE/100 g)**
Milk (control)	1012±210
E. durans 61	912±56
E. faecium 67	1265±112
E. faecium 75	1252±43
E. faecium 81	948±108
E. faecium 115	1143±97
E. durans 6363	1024±144
E. durans 6379	1255±86
E. durans 6380	940±84
E. durans 6413	869±132
E. durans 6451	1020±208
E. faecium 9683	1186±44
L. plantarum 83	2086±163*
L. acidophilus 94	1668±212*
L. casei 100	1066±104
L. rhamnosus 108	1841±91*
L. casei 116	1465±124*
L. brevis 122	1112±64
L. paracasei 2647	1342±110*
L. rhamnosus RH	2145±84*

Data represent average values ± standard deviation (SD).
**p<0.05 vs. control.*

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Table 2. *Induction factor (Is) of the E. coli MG1655 PSoxS-lux strain with the addition of lactobacilli-fermented milk supernatants with and without inducer (paraquat). The values of increased (↑) and decreased (↓) (Is) compared to (Is) of control are given in parentheses, %*

Strain		**Volume of supernatant in solution, %**			
		10	**1**	**0.1**	**0.01**
Control	no paraquat	0			
	with paraquat	25.2			
Milk	no paraquat	1.2	-1.4	1.1	1.0
	with paraquat	18.6 (26%↓)	20.9 (17%↓)	21.4 (15%↓)	22.9 (9%↓)
L. plantarum 83	no paraquat	0.9	1.1	-0.8	1.0
	with paraquat	2.3* (91%↓)	2.8* (89%↓)	7.6* (70%↓)	14.6* (42%↓)
L. acidophilus 94	no paraquat	0.3	0.7	0.2	0.9
	with paraquat	7.6* (70%↓)	9.1* (64%↓)	13.1* (48%↓)	19.9* (31%↓)
L. casei 100	no paraquat	-0.4	0	-0.3	0.4
	with paraquat	16.9 (33%↓)	18.9 (25%↓)	20.9 (17%↓)	23.2 (8%↓)
L. rhamnosus 108	no paraquat	1.3	0.4	0.9	-0.7
	with paraquat	8.3* (67%↓)	11.6* (54%↓)	12.9* (49%↓)	18.4* (27%↓)
L. casei 116	no paraquat	0.8	0.7	-0.4	0.8
	with paraquat	11.3* (55%↓)	13.9* (45%↓)	19.2* (24%↓)	22.7* (10%↓)
L. brevis 122	no paraquat	-0.5	0.5	1.1	-0.3
	with paraquat	17.1 (32%↓)	18.2 (24%↓)	20.7 (18%↓)	23.2 (8%↓)
L. paracasei 2647	no paraquat	1.4	-0.2	0.3	1.1
	with paraquat	16.1* (36%↓)	16.9* (33%↓)	20.4 (19%↓)	23.2 (9%↓)
L. rhamnosus RH	no paraquat	0.8	0.2	0.5	1.2
	with paraquat	4.0* (84%↓)	5.8* (77%↓)	7.3* (71%↓)	22.9* (38%↓)

**p<0.05 vs. paraquat + milk.*

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Table 3. *Induction factor (Is) of the E. coli MG1655 PSoxS-lux strain with the addition of enterococci fermented milk supernatants, with and without inducer (paraquat). The values of increase (↑) and decrease (↓) (Is) compared to (Is) of control are given in parentheses, %*

Strain	**Inducer**	**Volume of supernatant in solution, %**			
		10	**1**	**0.1**	**0.01**
Control	no paraquat	0			
	with paraquat	25.2			
Milk	no paraquat	1.2	-1.4	1.1	1.0
	with paraquat	18.6 (26%↓)	20.9 (17%↓)	21.4 (15%↓)	22.9 (9%↓)
E. durans 61	no paraquat	2.6	0.4	1.1	-0.3
	with paraquat	39.1** (55%↑)	27.3** (8%↑)	24.7 (2%↓)	23.2 (8%↓)
E. faecium 67	no paraquat	3.8*	1.2	0.6	0.8
	with paraquat	41.1** (63%↑)	27.7** (10%↑)	25.0 (1%↓)	22.8 (10%↓)
E. faecium 75	no paraquat	2.7*	0.9	-0.6	0.1
	with paraquat	31.2** (24%↑)	21.7 (14%↓)	21.4 (15%↓)	23.5 (7%↓)
E. faecium 81	no paraquat	3.1*	0.7	0.4	1.2
	with paraquat	35.1** (39%↑)	24.2 (4%↓)	20.7 (18%↓)	23.6 (6%↓)
E. faecium 115	no paraquat	3.0*	0.4	0.6	1.0
	with paraquat	38.1** (51%↑)	27.2** (8%↑)	24.0 (5%↓)	22.8 (9%↓)
E. durans 6363	no paraquat	2.9*	-0.7	1.1	0.3
	with paraquat	38.8** (54%↑)	28.3** (12%↑)	23.5 (7%↓)	23.1 (9%↓)
E. durans 6379	no paraquat	2.3	0.9	0.6	-0.3
	with paraquat	33.6** (33%↑)	21.4 (15%↓)	22.2 (12%↓)	22.5 (11%↓)
E. durans 6380	no paraquat	3.7*	0.2	0.8	1.2
	with paraquat	44.0** (75%↑)	29.2** (16%↑)	27.8** (10%↑)	21.6 (14%↓)
E. durans 6413	no paraquat	3.4*	0.7	0.8	0.9
	with paraquat	42.5** (69%↑)	29.6** (17%↑)	27.6** (10%↑)	22.1 (12%↓)
E. durans 6451	no paraquat	2.7*	0.6	0.6	0.9
	with paraquat	35.3 (40%↑)	22.6 (10%↓)	22.7 (10%↓)	23.4 (7%↓)
E. faecium 9683	no paraquat	3.2*	0.8	0.8	1.1
	with paraquat	45.5** (81%↑)	27.9** (11%↑)	22.8 (10%↓)	22.4 (11%↓)

**p<0.05 vs. (Is) of milk*
***p<0.05 vs. (Is) of paraquat + milk*

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Table 4. *Induction factor (Is) of the E. coli MG1655 pSoxS-lux strain with the addition of enterococci fermented milk supernatants with and without different inducers. The values of increase (↑) and decrease (↓) (Is) compared to (Is) of control are given in parentheses, %*

Inducer	No fermented milk	Milk	E. durans 6380	E. durans 6451	E. faecium 9683
No inducer	0	1.0	3.5*	2.8*	3.2*
Paraquat	25.4	19.1 (24%↓)	43.2** (70%↑)	43.4** (71%↑)	44.9** (77%↑)
Hydrogen peroxide	5.4	4.8 (11%↓)	7.5** (39%↑)	8.2** (52%↑)	7.7** (43%↑)
Dioxidine	5.1	4.3 (15%↓)	9.4** (84%↑)	9.5** (86%↑)	10.0** (96%↑)

**p<0.05 vs. (Is) of milk*
***p<0.05 vs. (Is) of inducer + milk*

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