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Lactobacillus casei suppresses *hfq* gene expression in *Escherichia coli* O157:H7

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Enterohaemorrhagic *Escherichia coli* O157:H7 is a zoonotic food and waterborne bacterial pathogen that causes a high hospitalization rate and life-threatening complications including seizures, cerebral oedema, haemolytic-uremic syndrome (HUS) and/or coma. The mortality associated with enterohaemorrhagic *E. coli* infections is due to the production and release of a Shiga toxin (Stx) by these bacteria [1]. The main regulator of this virulence gene is *hfq*, a bacterial RNA chaperone involved in virulence of an increasing number of bacterial pathogens [2]. A complex intestinal microflora provides protection against colonization by many pathogenic infectious agents. It has been hypothesized that foods fermented by lactobacilli help maintain a balance between lactobacilli and the indigenous intestinal flora [3]. The presence of lactobacilli in the gastrointestinal tract may suppress the growth of putrefactive and non-acid tolerant types of bacteria, thus reducing the amount of toxic substances generated [4]. Antimicrobial activity has been reported by co-culturing the symbiotic bacteria and pathogens in many studies. Bacteriocin-producing *Lactobacillus* spp provides protection against *E. coli* invasion during transit through in a dynamic model of the human stomach and small intestine [5]. Studies carried out both in culture media and foods have shown that bacteriocins produced by certain *Lactobacillus* spp can act synergistically antimicrobial activity [6]. Interestingly, *Lactobacillus* spp may simultaneously secrete organic acids and bacteriocins. Some studies have shown *Lactobacillus* spp to possess inhibitory activity towards the growth of pathogenic bacteria such as *E. coli* [7], but the changes in virulence genes by *Lactobacillus* spp against pathogens have not been studied yet. We hypothesized that *Lactobacillus casei* would exert a beneficial effect on *E. coli* by decreasing the virulence activity and growth rate of the latter.

E. coli O157:H7 PTCC 43889, which produces both Shiga toxins (Stx1 and Stx2), was grown in Luria-Bertani (LB) broth at 37°C for 24 h. *Lactobacillus casei* PTCC1608 was grown overnight at 37°C in MRS broth which had been purged of oxygen with nitrogen. Co-culture experiments whereby *L. casei* was incubated with *E. coli* in a variety of times (every 4 to 24 h, in 6 intervals) in Mueller Hinton broth, to determine whether *L. casei* was able to exert an inhibitory effect by bacteriosin or not. Total RNA from bacteria was extracted, and quantity and quality were determined using a NanoDrop ND-1000 spectrophotometer and electrophoresis on 1% agarose gel, respectively. For mRNAs reverse transcription, 1 µg of extracted RNA was reverse-transcribed using a universal hexamer primer. RNA and 1 µL dNTP and DEPC water were mixed and incubated at 65°C for 5 min and immediately transferred on ice. Then, 5 U of reverse transcriptase enzyme (MMLV), 4 µL 10x RT buffer, 2 U RNase inhibitor were added to the reaction and the volume of the solution was increased to 20 µL with DEPC water. Reverse transcription of mRNAs was performed at 25°C for 10 min and 42°C for 60 min. The reaction followed by an inactivation at 72°C for 5 min. The resulting cDNA was stored at −20°C until required. The Real-time PCR was performed to measure expression levels of target mRNAs using a SYBR Master Mix (Life Technologies) on a Bio-Rad IQ5 real-time PCR detection system according to specific 16SrRNA gene primers with 90 bp product (forward: 5-ACTCTGTTATTAGGGAAGAA-3 and reverse: 5-AACGCTTGCCACCTACGTAT-3). The Q-PCR was performed at 95°C for 30 s, followed by 45 cycles at 95°C for 5 s, 58°C for 30 s, and 72°C for 25 s. After completion of PCR cycling, melting curves were generated at 95°C to verify specificity. To generate standard curves, qPCR amplification of cDNA and their 10^{-1}–10^{-5} dilutions were carried out. The level of expression was calculated based upon the PCR cycle number (C_T). The endogenous controls 16S ribosomal were used for normalization of mRNAs expression level. Ct values were used to calculate relative expression by using of REST 9 software by the difference in the C_T values of the target RNAs after normalization to 16S ribosomal RNA. Relative quantification was represented by standard $2^{\Delta CT}$ calculations ($\Delta C_T = C_{T\text{-target gene}} - C_{T\text{-16S ribosomal}}$). Each reaction was performed in triplicate.

The results showed that *L. casei* have no effect on the growth of *E. coli* O157:H7 under co-cultured condition. The culture was repeated in Mueller

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Hinton agar, but an inhibitory effect was not shown. The result showed that *L. casei* could not produce bacteriocin against *E. coli* O157:H7, but in liquid media it could effect on growth rate. (Figure 1). The expression of *hfq* gene in *E. coli* O157:H7 was calculated based upon the PCR cycle number (C_T) and 16S ribosomal gene was used for normalization of mRNAs expression level. The results showed that *L. casei* down-regulated the *hfq* gene expression after 8 and 12 h of co-culture with *E. coli* O157:H7 ($p = 0.02$) (Figure 2). The *hfq* gene expression levels in *E. coli* O157:H7 samples in the presence of *L. casei* were variable between 0.133 and 1.414, and therefore on average, gene expression was 0.753 in comparison with *E. coli* singly. Indeed, it was shown that the amount of *hfq* gene expression is reduced in the presence of *L. casei* as time passed in 8 and 12 h.

Recent studies have identified an important role for *hfq* in pathogenesis for both gram-negative and gram-positive bacteria. *hfq* is part of the enterohaemorrhagic *E. coli* regulatory cascade [2]. It has shown that *hfq* is an important regulator of virulence traits in several bacterial species, including *E. coli* [8]. *hfq* promotes interactions between an sRNA and its target mRNA to regulate gene expression; however, *hfq* can function independently by influencing polyadenylation or translation of mRNAs [9]. Also, reported that *hfq* regulates genes encoding Stx. It is possible that *hfq* and sRNAs act to define a threshold for expression of *Stx* gene [10]. *hfq* plays an important role in fine-tuning of enterohaemorrhagic *E. coli* virulence gene expression. *hfq* synchronizes gene expression from the level of cell-to-cell signalling, to host attachment and colonization, to expression of Stx [11]. Deletion of *hfq* also caused decreased expression of the two-component system *qseBC*, which is involved in interkingdom signalling and virulence gene regulation in enterohaemorrhagic *E. coli*, as well as an increase in expression of *stx*2AB, which encodes the Shiga toxin [11]. Deletion of *hfq* affected transcription of many genes in nonpathogenic and pathogenic strains of *E. coli,* as well as pathogen-specific genes. They cause endothelial damage with

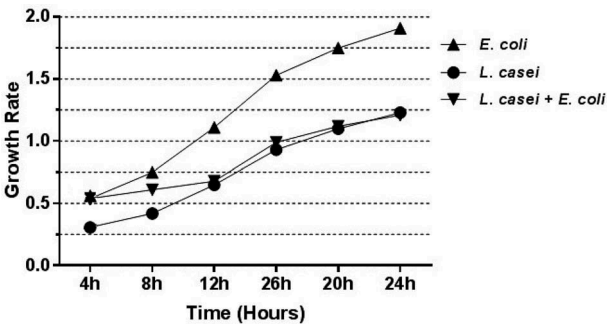

Figure 1. The growth curve of co-cultured bacteria. *L. casei* was co-cultured with *E. coli* in a variety of times.

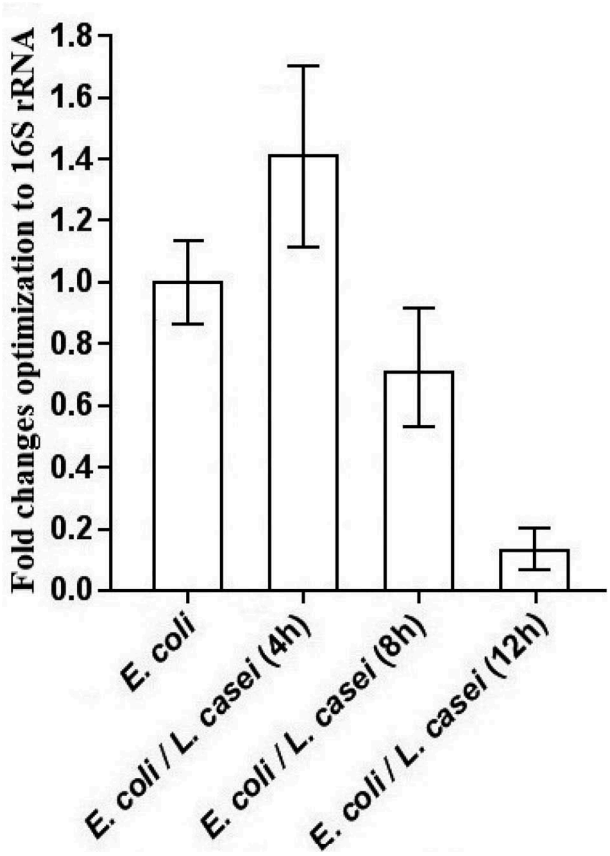

Figure 2. The expression of *hfq* in *E. coli* O157:H7 when co-cultured with *L. casei*.

consecutive systemic thrombotic microangiopathy, resulting in renal failure and haemorrhagic colitis [12]. These toxins are the main and serious food-poisoning outbreak caused by *E. coli* occurred in many countries [13]. Here, we studied the possibility effect of *lactobacillus* spp as an attractive candidate for suppressing *hfq*. In bacteria, the quorum sensing (QS) system controls and adjusts the expression of virulence factors, physiological activities, secondary metabolites production and even relationship with each other [14]. QS is related to production and releasing the small messenger molecules called autoinducers by microorganisms to their environment. As the microorganisms grow and increase, the amount of autoinducers production increases. When the concentration of these molecules reaches a proper limit or threshold, microorganisms understand the message and respond by expressing specific genes. Overall, the principles of QS include increasing the number of cells, the production of autoinducers to the threshold of stimulation and activation or inhibition of genes [15].

Our data show that *L. casei* can down-regulate *hfq* under co-cultured condition. Therefore, this could be new strategy to fight against pathogeneticity of pathogenic bacteria without any manipulation in their balances and population. However, further studies on effect of other bacteria on expression of *hfq* and other virulence factors are recommended.

This work represents an advance in biomedical science because it points to a mechanism of *L. casei* suppressing *E. coli* infections.

Disclosure statement

No potential conflict of interest was reported by the authors.

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