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Effect of Glucose in Removal of Microcystin-LR by Viable Commercial Probiotic Strains and Strains Isolated from Dadih Fermented Milk

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The removal of the cyanobacterial peptide toxin microcystin-LR at 4 and 37 °C by six commercial probiotic strains and *Lactobacillus plantarum* strains IS-10506 and IS-20506 isolated from dadih, Indonesian traditional fermented milk, was assessed in this study. The aim was to evaluate the main factors influencing the viability and metabolic activity of the probiotic strains, as well as their capacity to remove microcystin-LR. Both *L. plantarum* strains isolated from dadih, as well as *Bifidobacterium lactis* strains Bb12 and 420, were shown to be more resistant, and >85% remained viable in phosphate-buffered saline (PBS) solution for 48 h of incubation at either temperature, while the viability of the other four commercial bacteria decreased markedly over time. The effect of glucose on viability and removal of toxin was shown to be a strain-specific and strain-dependent property, but in general, the efficiency of microcystin-LR removal increased when glucose was added to the solution. A maximum removal of 95% was observed for *L. plantarum* strain IS-20506 (37 °C, 10^{11} colony-forming units mL^{-1}) with 1–2% glucose supplementation and 75% in PBS alone.

KEYWORDS: Microcystin-LR; probiotic; viability; glucose; metabolism

INTRODUCTION

In many eutrophic freshwaters, cyanobacteria frequently form toxic mass occurrences. Cyanobacteria produce a number of potent hepato- and neurotoxins collectively called cyanotoxins. Cyanotoxins show a potent acute hepatotoxicity and tumor-promoting activity and are therefore considered a significant public health problem worldwide. Microcystins are cyclic heptapeptide hepatotoxins, which are produced by several cyanobacterial genera. In addition to potent acute hepatotoxicity, microcystins are tumor promoters (*1*) and possible carcinogens (*2*). Toxic cyanobacteria are increasingly found in drinking water reservoirs, with cells or dissolved toxins entering the water supply (*3*). Cyanotoxins contaminate drinking water resources and raw materials for the food industry, including animal tissues and agricultural products. Microcystin-LR (MC-LR) has been regarded as the most common and the most toxic variant among approximately 80 microcystins, and a provisional guideline value of 1 $\mu g\ L^{-1}$ for MC-LR in drinking water has been issued by the World Health Organization (*4*). Exposure to microcystins has been associated with the illness and even death of animals and humans (*5–7*), and several incidences of the acute effects of hepatotoxins that cause toxic liver injury (*8*) or death (*9*) have been reported. Because the removal of toxins from water supplies is highly important, it is advantageous to identify bacteria capable of microcystin degradation.

A probiotic has been defined by the FAO-WHO as a "live microorganism which when administered in adequate amounts confers a health benefit on the host" (*10*). Previous studies have shown that specific strains of lactic acid bacteria and bifidobacteria are effective in removal of mycotoxins, such as ochratoxin A and aflatoxin B_1, from solution (*11–13*). Furthermore, lactic acid bacteria have been shown to be effective in the removal of heavy metals, such as cadmium and lead (*14*). Lyophilized strains of probiotic bacteria have also been shown to remove MC-LR from solution (*15*).

This study was designed to demonstrate the ability of specific commercial strains of lactobacilli and bifidobacteria and the *Lactobacillus plantarum* strains isolated from traditionally fermented dadih to remove the cyanobacterial peptide toxin MC-LR from water solutions. Dadih, a yogurtlike product, is an Indonesian traditional fermented product from West Sumatra, which is spontaneously fermented from fresh raw buffalo milk in bamboo tubes and capped with banana leaves (*16*). Different

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bacteria, mainly lactobacilli (*L. plantarum, L. casei*, and others), enterococci (*E. faecium*), and some pediococci can be found in dadih (*17, 18*). *L. plantarum* is a versatile strain that is present in a range of environmental niches including dairy, meat, and vegetable fermentations (*19*). Studies on potential probiotic properties of indigenous lactic acid bacteria isolated from dadih have demonstrated antimutagenic, mutagen-binding, and cholesterol-binding properties, as well as acid and bile tolerance, good adhesion properties, and antipathogenic properties (*18, 20–23*).

In a recent study, it was shown that viability is a requirement for efficient removal of MC-LR from solution. Nonviable bacteria inactivated by different treatments were not able to remove MC-LR as efficiently (*24*). The aim of this study was to investigate the main factors influencing the metabolic activity and enhancing the viability of commercial probiotic strains and *L. plantarum* strains isolated from dadih in removal of MC-LR. The specific aims were to assess the impact of temperature, the importance of cell viability, the role of glucose in activating the metabolism of the probiotic bacteria, and the effect of the bacterial cell density in toxin removal.

MATERIALS AND METHODS

Chemicals. Methanol [high-performance liquid chromatography (HPLC)-grade] and acetonitrile (HPLC S-grade) were from Rathburn (Walkerburn, United Kingdom). Trifluoroacetic acid (TFA) was from Fluka (Buchs, Switzerland). Water was purified to 18.2 MΩ cm on a Milli-Q Synthesis system (Millipore, Molsheim, France). MC-LR test solutions were prepared in 0.01 M phosphate-buffered saline (PBS) buffer (130 mM sodium chloride, 10 mM sodium phosphate, pH 7.2) with or without the addition of glucose (J.T. Baker, Deventer, The Netherlands).

Microcystins. MC-LR was extracted from a culture of *Anabaena* sp. 90 (originating from the culture collection of Prof. Kaarina Sivonen, University of Helsinki, Finland) and purified by HPLC as described earlier (*25*). Ten micrograms of MC-LR was dissolved in 1 mL of PBS buffer, and appropriate dilutions were made.

Bacterial Strains. Six commercial probiotic and two dadih lactic acid bacterial strains were tested for their microcystin-removal capacity. The commercial strains were *Lactobacillus rhamnosus* GG (ATCC 53103, Valio Ltd., Helsinki, Finland), *L. rhamnosus* LC-705 (Valio Ltd.), *L. plantarum* Lp-115 (Danisco Inc., Madison), *Bifidobacterium longum* 46 (DSM 14583), *Bifidobacterium lactis* 420 (Danisco Deutschland Gmbh, Niebüll, Germany), and *B. lactis* Bb12 (Chr. Hansen Ltd., Hørsholm, Denmark), and they were obtained in commercial lyophilized form. The indigenous dadih strains were *L. plantarum* strains with the identification numbers IS-10506 and IS-20506 (Gene Bank Accession numbers DQ860148 and DC860149, respectively) from the University of Turku culture collection.

The bacteria were cultured in deMan−Rogosa−Sharpe (MRS) broth (Oxoid, Hampshire, United Kingdom) (MRS supplemented with 0.05% w/v cysteine−HCl for *Bifidobacteria*) for 18 h at 37 °C, harvested by centrifugation (3200*g*, 4 °C, 20 min), and washed twice with PBS buffer. The cells were then suspended in PBS with or without glucose (1, 2, or 3%) to reach a final cell density of approximately 10^{10} colony-forming units (CFU) mL^{-1}. Glucose was added in PBS as a bacterial nutrient. Total viable counts of bacterial suspensions were determined prior to incubation (control, time 0 h) by flow cytometry.

Plate Counts. Viable counts (CFU mL^{-1}) were obtained by plate counting from bacterial suspensions after 0, 24, and 48 h of incubation in PBS containing 100 $\mu g\ L^{-1}$ MC-LR. The bacterial suspensions were serially diluted in PBS buffer, and appropriate dilutions were spread on MRS agar plates (Oxoid, Hampshire, United Kingdom) (MRS agar supplemented with 0.05% w/v cysteine−HCl for *Bifidobacteria*) and incubated for 48 h at 37 °C (anaerobic conditions for *Bifidobacteria*; 10% H_2, 10% CO_2, and 80% N_2; Concept 400 anaerobic chamber, Ruskinn Technology, Leeds, United Kingdom). All plate counts were determined in three independent experiments, and each assay was performed in triplicate.

Fluorescent Counts. Counts of total, viable, and dead bacteria were obtained by the use of LIVE/DEAD BacLight Bacterial Viability Stain Kit (Molecular Probes, Eugene, OR). The viability kit stain mixture, used for total counts of bacterial population, distinguished live bacterial cells from dead by means of membrane integrity and increased the efficiency of staining to indicate viability (*26*).

Bacterial cells were washed with PBS buffer, and 10 μL was mixed with 1.5 μL of a mixture of SYTO9 and propidium iodide (PI) (1:1), nucleic acid stains from a LIVE/DEAD BacLight. The samples were then vortexed and incubated in darkness for 15 min at room temperature according to the manufacturer's instructions. Immediately prior to analysis, 10 μL of fluorospheres (Molecular Probes) was added to each sample to obtain the absolute bacterial cell count.

Flow cytometric analysis was performed using a BD FACSCalibur flow cytometer (Becton, Dickinson and Co., United States) with an air-cooled argon ion laser (488 nm at 15 mW). This standard instrument was equipped with two light scatter detectors that measure forward (FSC) and side scatter (SSC) and two fluorescence detectors (FL1, 525 nm; FL3, 620 nm). Data were stored as list mode files and analyzed off-line using the System II V.3 software (Beckman Coulter). Ideally, healthy (live) bacteria with intact plasma membranes fluoresced green, and the dead or injured cells with compromised membranes fluoresced red. In accordance with the manufacturer's instructions, all green cells (SYTO9) were considered viable, and red cells (PI) were considered dead. Viability was determined in two independent experiments, and each assay was performed in duplicate.

The percentages of viability were calculated comparing the total viable counts before incubation ($t = 0$, 100% viability) with the viable cells labeled with SYTO9 (green color) obtained after 24 and 48 h of incubation at 4 and 37 °C in different concentrations of glucose.

Microscopy. Visual inspection of the viability of bacteria (for *L. rhamnosus* GG) during incubation with MC-LR at 37 °C in absence or presence of glucose (0 or 1%) was determined by microscopy. An Olympus epifluorescence microscope BX50 (Olympus, Tokyo, Japan) with filter U-MWIB (excitation, 460–490 nm; emission, 515–700 nm) was used. Images were recorded with a digital camera (model DP-10; Olympus, Hamburg, Germany): green bacteria, viable; red bacteria, nonviable; and orange bacteria, injured.

Microcystin Removal Assay. One milliliter of the bacteria suspended in PBS at a concentration of approximately 10^{10} CFU mL^{-1} with or without the addition of glucose (1, 2, or 3%) together with 100 $\mu g\ L^{-1}$ MC-LR were incubated in 1.5 mL borosilicate glass chromatographic vials under continuous reciprocal shaking, 120 rotations per minute (Certomat WR, B. Braun, Melsungen, Germany) at 4 or 37 °C for different times (0, 18, 24, and 48 h). After incubation, subsamples were taken and centrifuged (12000*g*, 10 min, room temperature) in 300 μL borosilicate glass chromatographic inserts. The supernatants were analyzed to determine the residual MC-LR concentration by HPLC as compared with a 100 $\mu g\ L^{-1}$ MC-LR control in PBS.

HPLC Analysis. MC-LR was quantified by HPLC on a Purospher STAR RP-18 endcapped column (55 mm × 4 mm, 3 μm particles; Merck, Darmstadt, Germany) as described earlier (*27*). The mobile phase consisted of a gradient of 0.05% aqueous TFA (solvent A) and 0.05% TFA in acetonitrile (solvent B) with the following linear gradient program: 0 min 25% B, 5 min 70% B, 6 min 70% B, and 6.1 min 25% B. The injection interval was 10 min, the injection volume was 30 μL, the flow rate was 1.0 mL min^{-1}, and the column oven temperature was 40 °C. The HPLC-DAD system consisted of a Merck-Hitachi (Darmstadt, Germany) LaChrom series instrument with a photodiode array UV detector operated at 238 nm. MC-LR eluted at ca. 3.9 min.

Statistics. Statistical analysis was performed using the *t* test. The probability level of 5% ($p = 0.05$) was used to indicate the significance.

RESULTS

The results show that all tested strains were able to remove MC-LR from aqueous solutions, but differences in the elimination efficiency of the strains were demonstrated. **Figure 1** shows

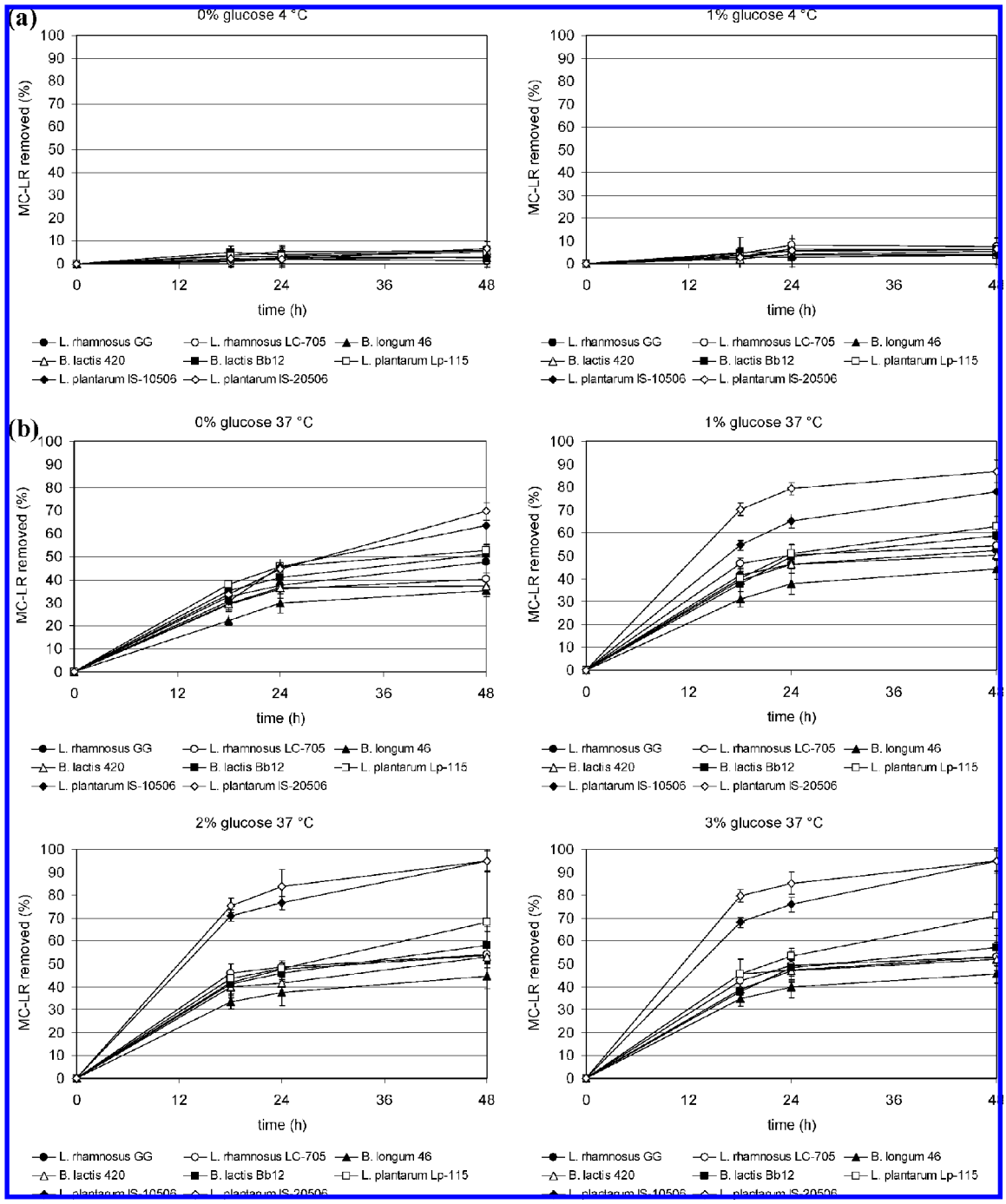

Figure 1. Percentage of MC-LR removed by freshly cultured bacteria incubated in PBS containing glucose. Initial MC-LR concentration, 100 μg L^{-1}; incubation times, 0, 18, 24, and 48 h; average ± SD, $n = 3$; (**a**) 4 °C and 0 or 1% glucose and (**b**) 37 °C and 0, 1, 2, or 3% glucose.

the removal percentages of MC-LR for all tested strains at 4 °C (**Figure 1a**) and 37 °C (**Figure 1b**) when suspended in PBS alone, which cannot provide any nutrients to the bacteria, and added with glucose. The temperatures were chosen to test the influence of bacterial metabolic activity on the removal efficiency. A temperature of 4 °C was chosen as the temperature at which the probiotic bacteria are metabolically inactive. A temperature of 37 °C is the optimal growth temperature for the commercial probiotic bacteria, while the optimal growth temperature for the *L. plantarum* strains is 30 °C (*28*). In addition, the metabolic activity of the bacteria is influenced by the glucose addition.

Practically no removal of MC-LR could be observed at 4 °C, whereas at 37 °C all bacterial strains tested were able to remove MC-LR (**Figure 1**) although the removal efficiency was dependent on the strain and on glucose addition. The results suggest that bacterial metabolic activity has an important role in toxin removal because at 37 °C, when the bacteria are able to grow, the removal percentages were highest ($p < 0.05$) for all tested strains (**Figure 1b**). The best MC-LR removal percentages were obtained at 37 °C using *L. plantarum* strains isolated from dadih fermented milk.

Furthermore, the glucose addition improved the removal efficiencies of all tested strains at 37 °C by enhancing both the removal rate and the amount of MC-LR removed after a 48 h incubation (**Figure 1b**). In general, glucose had a positive effect on the removal abilities, with higher removal percentages observed with the addition of 1% glucose. The addition of 2 or 3% glucose did not significantly improve or affect the removal

Table 1. Counts on MRS Agar from Cell Suspensions Incubated in PBS at 37 °C[a]

strain	CFU mL^{-1} 0 h	24 h	48 h
L. rhamnosus GG	1.7×10^9	2.9×10^6	6.3×10^5
L. rhamnosus LC-705	2.0×10^{10}	2.5×10^7	1.4×10^7
B. longum 46	4.3×10^{10}	1.3×10^6	1.1×10^5
B. lactis 420	1.5×10^8	2.0×10^5	4.6×10^4
B. lactis Bb12	5.7×10^9	6.8×10^6	7.9×10^5
L. plantarum Lp-115	8.3×10^9	6.0×10^6	7.1×10^5
L. plantarum IS-10506	1.4×10^{11}	2.0×10^9	8.1×10^7
L. plantarum IS-20506	3.2×10^{11}	3.0×10^9	5.1×10^8

[a] All counts were significantly different as compared to control time 0 h. Initial MC-LR concentration, 100 μg L^{-1}; incubation times, 24 and 48 h.

further. At 4 °C, glucose did not have any effect on the removal efficiency (**Figure 1a**).

In addition, the viability of the bacteria during incubations of 24 and 48 h with MC-LR was of interest, as it has been shown in a recent study that viability is a requirement for efficient removal of MC-LR from solution (*24*). As the viability of bacteria in PBS buffer was shown to decrease over time during a 48 h incubation at 37 °C (**Tables 1** and **2**, **Section B**), glucose was added as a source of energy to the buffer solution to investigate its affect on viability.

The viability of all bacterial strains tested was analyzed by both plate counting (**Table 1**) and the LIVE/DEAD staining kit using flow cytometry (**Table 2**). The effect of glucose on the cell viability is shown in **Table 2**. At 4 °C, 100% of the bacteria stayed viable during an incubation of 48 h and the addition of 1% glucose did not affect the viability of the cells (**Table 2**, **Section A**). At 37 °C, glucose had varying effects on the viability of the bacteria, depending on the probiotic strain tested (**Table 2**, **Section B**). In general, the viability of all bacterial strains tested decreased at 37 °C in the presence of glucose, but the behavior was strain-dependent. At 37 °C, the metabolic activity rate is higher in the presence of glucose and the viable cells may experience stress as shown by the decrease of viability. For some of the strains, such as *L. rhamnosus* GG and *B. longum* 46, the viability decreased dramatically as a result of the addition of glucose, whereas for other strains (*L. plantarum* IS-10560 and IS-20560 and *B. lactis* Bb12 and 420) glucose did not significantly affect the viability within 24 h of incubation but viability decreased after 48 h of incubation in the presence of glucose (**Table 2**, **Section B**). The *B. lactis* strains Bb12 and 420 showed viabilities similar to those of the dadih strains at 37 °C (**Table 2**, **Section B**), but the removal percentages observed were lower. This suggests that strain-specific characteristics, other than viability, also play a role in the removal of MC-LR.

The total bacterial population was enumerated by counting green (SYTO9) and red microorganisms (PI) using flow cytometry. The results were also confirmed by an epifluorescence microscope (**Figure 2**). The microscopic images showed that in the absence of glucose most of the cells stayed viable until 24 h of incubation, while the presence of glucose reduced cell viability markedly already after 24 h of incubation. The images also support the conclusion that the bacteria become stressed as a result of higher metabolic activity when glucose is added. Consequently, the cells die faster in the presence of glucose. All three techniques (plate counting, flow cytometry, and microscopy) demonstrated that cell viability decreased during incubation and with an increasing concentration of glucose.

Furthermore, the influence of the bacterial concentration on MC-LR removal was assessed. **Figure 3** shows the effect of the bacterial cell density on the removal of MC-LR. All probiotic strains showed a similar tendency in MC-LR removal with increasing removal percentages observed with increasing bacterial cell density. The probiotic strains isolated from dadih showed high removal abilities also at lower bacterial concentrations (10^9-10^{10} CFU mL^{-1}) than the commercial probiotics, but with higher bacterial cell densities, the removal abilities were similar with all strains. In addition, dadih strains of *L. plantarum* (IS-10506 and IS-20506) were shown to have higher removal abilities as compared to the commercial *L. plantarum* strain Lp-115, which was shown to behave similarly to the other commercial strains.

DISCUSSION

The removal of MC-LR by different probiotic strains, both commercial strains and natural strains isolated from dadih fermented milk, was investigated under different conditions, with different temperatures and bacterial cell densities in the absence or presence of glucose, to find the optimal conditions for toxin removal. Bacterial viability was assessed by traditional plate counting as well as flow cytometry and microscopy. The correlation between plate recount and flow cytometry has previously been found to be good (*29, 30*). The results of this study show that natural isolated strains from dadih, an Indonesian traditional fermented product, were able to remove MC-LR efficiently up to 48 h at 37 °C (**Figure 1**) when incubated in PBS with or without glucose, while the removal efficiencies of the commercial strains started to decrease after 24 h of incubation. This could be explained by the different viability properties of the bacterial strains observed during incubation in PBS solution, which could affect their removal capacities. Viable cell counts estimated by plating onto selective media (**Table 1**) were lower than those obtained by direct microscopic counting (**Figure 2**) or flow cytometry (**Table 2**, **Section B**). This can be explained by the presence of bacteria that have a damaged or altered integrity of the membrane. Consequently, they cannot grow in specific cultivation media but still remain viable.

For the dadih strains, a clear improvement on the removal ability was observed by the addition of glucose to the solution, but the increase was not so marked for the other strains. Of the commercial strains, *B. lactis* Bb12 and 420 showed similar viabilities when compared to the dadih strains, but their MC-LR removal efficiencies were significantly lower. This suggests that in addition to viability, other strain-specific properties also affect the removal efficiency.

The results demonstrate that both viability and metabolic activity play an important role in the removal of MC-LR. Supplementation of glucose provides energy to the bacteria, and thereby, the MC-LR removal efficiencies also increase. During incubation, the bacterial strains consume glucose and as a consequence produce lactic acid, which in turn causes cell stress as a result of the decrease in pH. In this study, the bacterial cells removed MC-LR from solution faster in the presence of glucose but cell death also occurred faster. In the previous study by Nybom et al. (*24*), the MC-LR removal efficiencies of probiotic strains were compared at pH 3 and 7. The removal was more efficient at neutral pH, which suggests that the bacterial cells suffer from the low pH, thereby also affecting the MC-LR removal.

Table 2. Percentage of Viable Cells Incubated at 4 °C in PBS Containing Glucose (0 or 1%) (Section A) and Percentage of Viable Cells Incubated at 37 °C in PBS Containing Glucose (0, 1, 2, or 3%) (Section B)[a]

Section A

	0% glucose		1% glucose	
strain	24 h	48 h	24 h	48 h
L. rhamnosus GG	99.2 ± 1.4	99.4 ± 1.0	99.1 ± 1.4	100.2 ± 2.4
L. rhamnosus LC-705	100.6 ± 2.1	100.8 ± 0.3	100.3 ± 1.2	100.5 ± 1.1
B. longum 46	100.5 ± 0.6	100.8 ± 0.5	99.4 ± 0.9	100.4 ± 0.7
B. lactis 420	101.5 ± 2.2	100.3 ± 2.1	99.1 ± 2.2	99.0 ± 3.0
B. lactis Bb12	99.3 ± 0.8	100.3 ± 3.2	102.5 ± 2.1	104.5 ± 2.1
L. plantarum Lp-115	99.5 ± 0.9	99.2 ± 1.6	100.1 ± 0.6	99.8 ± 3.3
L. plantarum IS-10506	99.4 ± 2.6	96.8 ± 2.0	98.8 ± 2.6	99.6 ± 1.7
L. plantarum IS-20506	99.2 ± 3.0	99.4 ± 1.7	99.1 ± 1.3	100.2 ± 0.7

Section B

	0% glucose		1% glucose		2% glucose		3% glucose	
strain	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
L. rhamnosus GG	88.2 ± 0.2	34.1 ± 0.2	0.2 ± 0.1	0.8 ± 0.3	10.8 ± 0.5	0.8 ± 0.3	15.1 ± 0.3	0.2 ± 0.1
L. rhamnosus LC-705	73.8 ± 0.2	74.9 ± 0.5	0.7 ± 0.2	0.1 ± 0.0	13.2 ± 0.3	0.1 ± 0.1	11.8 ± 0.6	0.1 ± 0.1
B. longum 46	99.5 ± 0.5	72.6 ± 0.6	15.6 ± 0.5	2.4 ± 0.3	38.0 ± 0.2	1.7 ± 0.2	28.3 ± 0.6	2.5 ± 0.2
B. lactis 420	97.8 ± 0.3	94.5 ± 0.4	83.2 ± 0.3	74.4 ± 0.5	76.2 ± 0.6	77.3 ± 0.3	47.4 ± 0.8	37.9 ± 0.3
B. lactis Bb12	99.8 ± 0.5	86.5 ± 0.2	77.9 ± 0.2	52.2 ± 0.2	79.2 ± 0.5	27.6 ± 0.5	61.9 ± 0.5	24.0 ± 0.2
L. plantarum Lp-115	73.8 ± 0.2	74.9 ± 0.5	0.7 ± 0.2	0.1 ± 0.0	13.2 ± 0.3	0.1 ± 0.1	11.8 ± 0.6	0.1 ± 0.1
L. plantarum IS-10506	99.6 ± 0.3	97.1 ± 0.3	88.5 ± 0.2	59.5 ± 0.2	11.6 ± 0.3	0.5 ± 0.1	6.1 ± 0.2	0.1 ± 0.1
L. plantarum IS-20506	88.4 ± 0.2	85.1 ± 0.2	78.3 ± 0.5	40.5 ± 0.3	26.5 ± 0.5	0.2 ± 0.0	12.7 ± 0.2	0.2 ± 0.1

[a] Viability determined by flow cytometry. Initial MC-LR concentration 100 μg L^{-1}, incubation times 24 and 48 h, average ± SD, $n = 2$. Viability at 0 $h = 100\%$.

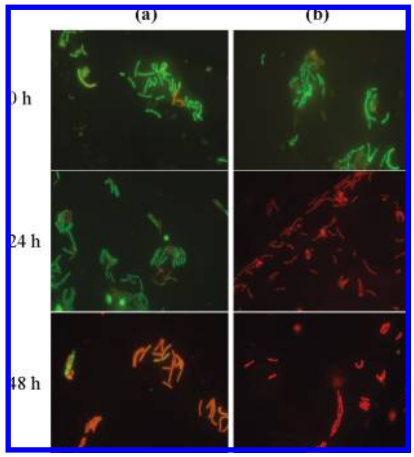

Figure 2. Viability of bacteria (*L. rhamnosus* GG) after 0, 24, and 48 h of incubation at 37 °C in the absence or presence of 1% glucose determined by microscopy. Green bacteria, viable; red bacteria, nonviable; and orange bacteria, injured; (**a**) 0% glucose and (**b**) 1% glucose.

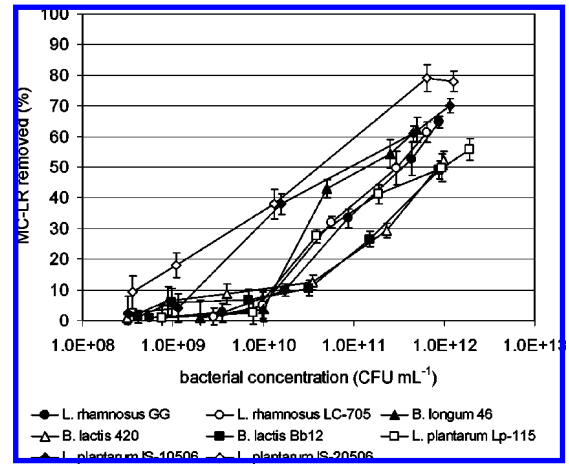

Figure 3. Effect of bacterial concentration on removal of MC-LR. Initial MC-LR concentration, 100 μg L^{-1}; incubation time, 24 h; temperature, 37 °C; and average ± SD, $n = 3$.

Differences between the bacterial strains could be observed both in their microcystin removal properties and in the viability of the bacteria during incubation. In general, the dadih probiotic strains were more resistant and stayed viable longer. As a result also, the removal percentages observed for *L. plantarum* IS-10506 and IS-20506 in the presence of glucose were higher than for the commercial probiotics. Also, other strain-dependent properties influencing the removal efficiencies were observed. The commercial *L. plantarum* strain Lp-115 was shown to behave more similar to the other commercial strains than with the *L. plantarum* strains isolated from dadih.

The results of this study reveal that cell viability, bacterial cell density, cell culturability, and metabolic activity play important roles in the removal of MC-LR. As a consequence of active metabolism, the viable cells become exhausted and stressed. Consequently, besides resulting in high removal ability, supplementation of glucose also resulted in decreased viability. In response to lack of energy or stress conditions, many bacteria are able convert into a viable but nonculturable condition (VBNC). **Table 1** shows that the dadih *L. plantarum* IS-10506 and IS-20506 strains and the *B. lactis* Bb12 and 420 are more resistant to unfavorable conditions, shown by the higher amount of culturable cells. Bacteria that have reached the VBNC state are unable to grow in conventional media but still maintain their membrane integrity and indicators of metabolic activity (*31–34*). In other words, they enter an active but nonculturable state. This may be potentially reversed by provision of nutritionally rich culture media and optimal growth conditions (*35, 36*). The results of this study suggest that the metabolic activity of the bacteria is maintained

functional in the cells, which can be detected by the enhanced removal of MC-LR at 37 °C in the presence of glucose (**Figure 1b**). Furthermore, the microscopic images support the fact that glucose enhances the metabolic activity of the cells, and thereby, the cells become stressed and cell death occurs sooner.

In conclusion, efficient toxin removal was observed for *L. plantarum* strains IS-10506 and IS-20506 and also for the commercial probiotic strains. The most efficient removal percentages were obtained at 37 °C with glucose supplementation, providing evidence for probable active metabolic elimination of MC-LR by probiotic bacteria. Probiotic bacteria may consequently have applications both in biological decontamination of microcystin-containing water and in the elimination of microcystins in the gastrointestinal tract.

ABBREVIATIONS USED

B., *Bifidobacterium*; CFU, colony-forming units; HPLC, high-performance liquid chromatography; *L.*, *Lactobacillus*; MC-LR, microcystin-LR; MRS, deMan−Rogosa−Sharpe broth; PBS, phosphate-buffered saline; PI, propidium iodide; TFA, trifluoroacetic acid; VBNC, viable but nonculturable.

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