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Bioavailability

Selenite-stress selected mutant strains of probiotic bacteria for Se source production

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ABSTRACT

Selenium deficiency is a major health problem worldwide for about 1 billion people. Bacterial cells usually possess low tolerance to selenite stress and also low ability to reduce high concentrations of toxic selenite. Here, high tolerance to selenite and selenium bioaccumulation capability were developed in mutated clones of probiotic and starter bacteria including *Enterococcus faecium*, *Bifidobacterium animalis* ssp. *lactis*, *Lactobacillus casei* and *Lactococcus lactis* ssp. *lactis* by food-level strain development process and clone selection. All mutant clones possessed increased glutathione concentration and glutathione reductase activity. The selenite treatment increased further these values in *L. casei* mutant strain pointing at a different selenite reduction pathway and/or stress response in this organism. Considerable conversion of selenite to cell bound selenium forms with a concomitant high biomass production was detected in *E. faecium* and *B. animalis* ssp. *lactis* cultures. Possible application of these strains as food and feed supplements is under investigation.

Introduction

Selenium is a micronutrient for all organisms and its deficiency is a major health problem for about 1 billion people worldwide [1]. Selenium status of people varies by country: intakes are usually high in North America and Japan, varies in China, while lower in Europe (especially in Eastern Europe) and Australia. Low selenium status is associated with poor immune function, oxidative stress and increased risk of mortality. Selenium has also been linked to an increased risk of cancer and to various neurodegenerative and cardiovascular diseases [2]. Selenium is needed by human brain functions, even at the expense of other tissues, and its shortage can also cause cognitive decline. High selenium status has antiviral effect *e.g.* against HIV and affects thyroid functions [3].

Selenium supplements contain selenium in different chemical forms. In the majority of supplements, the selenium is present as selenomethionine produced mainly by yeast [4]. However, in multivitamin preparations, weight-loss products, protein mixes, infant formulas and animal feed, sodium selenite and sodium selenate are usually supplemented [5]. As selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are highly water soluble ions, at high concentration they are toxic but less toxic than selenide (Se^{2-}) and having tendency to bioaccumulate [6]. Both the bioreduction of SeO_3^{2-} to nontoxic and biologically available elementary selenium (Se^0) and the incorporation of this transition metal into organic selenium compounds (mainly selenocystein) have been demonstrated in bacterial cultures (*e.g.* [7,8]), while, the reduction of SeO_4^{2-} could not be utilized for this purpose in lactic acid bacteria (*e.g.* [9]).

Several different mechanisms have been proposed to explain the biological reduction of SeO_3^{2-} although none is without controversy [10]. Glutathione (GSH) has long been suspected to be involved in selenium metabolism by selenodiglutathione (GS-Se-GS) formation [10]. GSH is a low molecular weight thiol antioxidant which has role in the maintenance of intracellular redox homeostasis to protect the cells against oxidative damage [11]. It is widely distributed in Gram negative organisms, but presents only sporadically in Gram positive bacteria [12]. Most of the biological functions of glutathione are mediated by the conversion of reduced glutathione (GSH) to its oxidized form (GSSG) by glutathione peroxidase and transformation of GSSG back to GSH by glutathione reductase (GR) with simultaneous oxidization of NADPH to $NADP^+$,

Abbreviations: DTNB, 5,5′-dithobis-(2-nitrobenzoic acid); GR, glutathione reductase; GSH, glutathione; TEA, triethanolamine.

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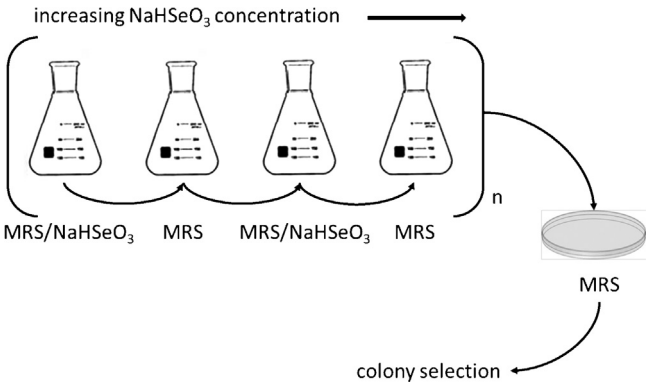

Fig. 1. Schematic presentation of development and selection of selenite resistant bacterial clones. The tested bacteria were inoculated into MRS media supplemented stepwise with increased $NaHSeO_3$ concentrations (0–10,000 mg L^{-1}). The final clone selection was done on the basis of the intensity of growth.

which is reduced by oxidoreductases, and this mechanism maintains cellular forms and levels of GSH [11].

In the present work mutants with high selenite tolerance and selenium accumulation were selected from well-known probiotic bacterium cultures, including *Enterococcus faecium* W54, *Lactococcus lactis* ssp. *lactis* R703, *Bifidobacterium animalis* ssp. *lactis* BB12 and *Lactobacillus casei* 431. These organisms are always preferred for biotechnological application (reviewed in [13]); however, have not been developed for selenite reduction. Characterization of growth and optimization of the cultivation were done. The possible correlation of the GSH level and the GR activity to selenium accumulation were also demonstrated and discussed.

Materials and methods

Medium and strains

E. faecium W54 (Ef; from ProGastro, Winclove Bio Industries, Amsterdam, The Netherlands), *L. lactis* ssp. *lactis* R703 (R703; Chr. Hansen A/S, Denmark), *B. animalis* ssp. *lactis* BB12 (BB12; Chr. Hansen A/S, Denmark), *L. casei* 431 (*L. casei*; Chr. Hansen A/S, Denmark) were used in the experiments. The bacterial strains were cultivated in de Man, Rogosa and Sharpe (MRS) [14] medium {containing 10 g L^{-1} proteose peptone, 8 g L^{-1} meat extract, 4 g L^{-1} yeast extract, 20 g L^{-1} glucose, 5 g L^{-1} sodium acetate, 2 g L^{-1} triammonium citrate, 0.2 g L^{-1} magnesium sulphate, 0.05 g L^{-1} manganese sulphate, 2 g L^{-1} dipotassium hydrogen phosphate, 1 g L^{-1} Polysorbate 80 (OXOID, UK) pH 6.8}.

Selection of selenite tolerant mutant strains

Strains were inoculated into MRS medium (pH 6.8) supplemented first with 0.331 mmol L^{-1} selenium (50 mg L^{-1} $NaHSeO_3$). After 24 h cultivation time, the cultures were inoculated into MRS without $NaHSeO_3$. Afterward, the concentration of selenite was increased up to 10,000 mg L^{-1} $NaHSeO_3$ (66.24 mmol L^{-1} final Se concentration) step-wise in each round of the transfers (inoculation into MRS with and without $NaHSeO_3$) (Fig. 1). The $NaHSeO_3$ stock solution (45 g L^{-1}) was sterilized by filtration and applied immediately.

Cultures with the highest selenite tolerance were pour-plated onto solid MRS medium, and single mutant colonies of each strain were chosen. Following the differential characterization protocol of lactic acid bacteria the mutant clones were tested, and also were identified with API®/rapid ID32 Strep test (bioMérieux, France) according to the instructions of the manufacturer using fresh bacterial cultures cultivated on MRS plates. Reading of the test strips was evaluated with miniAPI (bioMérieux, France) equipment.

Differential characterization of the parental and mutant strains

The growth was tested in MRS medium after 48 h cultivation time at different pHs (pH 6.8, pH 4.4 and 9.6) and temperatures (10 °C, 45 °C), and in the presence of high (6.5%) NaCl concentration. The morphology and catalase activity of the cells were also recorded. The catalase activity was tested in the H_2O_2 drop test, where the appearance of bubbles indicated catalase positive cells.

Characterization of growth of the selenite tolerant mutant strains

The mutant strains were inoculated into 10 mL liquid MRS aliquots, and after 16 h incubation at 32 °C, 10 μL inoculums (cell suspensions with 8 McFarland units cell density) were loaded to 200 μL aliquots of MRS media in microtiter plates. The growths of the strains were measured at different pHs (pH 5.97, pH 6.79, pH 8.01, pH 8.96) and temperatures (28 °C, 32 °C or 37 °C). The cell density was checked spectrophotometrically at λ = 630 nm. The growth was tested also with 100 mg L^{-1} and 200 mg L^{-1} sodium hydrogen selenite supplementation. All experiments were repeated three times.

Selenium production of the mutant strains

Bacteria were cultivated in 10 mL MRS medium for 16 h at 32 °C. From the culture media, 100 μL aliquots of the cultures (cell suspensions with 8 McFarland units cell density) were inoculated into 50 mL MRS medium supplemented with 600 mg L^{-1} $NaHSeO_3$. After 24 h cultivation, bacterial cells were centrifuged (6500 rpm, 10 min), the pellets were washed twice with phosphate buffered saline (PBS) and were frozen at −20 °C. After lyophilization, dry cell mass and selenium content of the biomass were determined.

The lyophilized samples were refilled to 1 mL with cc. HNO_3 and the samples were digested first with 3 mL ~~cm^{-3}.~~ HNO_3 at 120 °C for 50 min then with 2 mL 30% H_2O_2 at 120 °C for 50 min again. After cooling back to room temperature, the digested samples were filtered and adjusted to 10 mL with deionized water and stored in closed tubes. Each sample was analyzed in triplicates for selenium content on a flame emission atomic absorption spectrometer (Thermo Fischer Scientific, iCE 3000 Series AA Spectrometer, SOLAAR House, Cambridge, United Kingdom) and mean values of the selenium contents were calculated.

Total GSH and glutathione reductase assay

Bacteria were cultured in 10 mL MRS medium for 16 h at 32 °C. Following that, 50 mL aliquots of the MRS medium, which was supplemented with 600 mg L^{-1} $NaHSeO_3$, were inoculated with 100 μL cell suspensions of 8 McFarland units cell density. The cultures were grown for 24 h and the biomass was harvested by centrifugation (6500 rpm, 10 min). The cells were washed twice with 1 mL phosphate buffered saline (PBS) and were re-suspended in 500 μL 20 mmol L^{-1} TEA-HCl, pH 7.6, also containing 0.1 mmol L^{-1} EDTA. The cells were broken by sonication (five 30 s pulses with 30 s cooling periods) performed on ice. Crude homogenates were clarified by centrifugation and 20 μL of 5% sulphosalicylic acid was added to 200 μL aliquots of the supernatants to precipitate protein, which was removed by centrifugation (10,000 rpm, 10 min).

Before total GSH determination, 10 μL TEA was added to 200 μL sample, and the mixture was kept at 4 °C for 1 h. Reaction mixtures contained 125 mmol L^{-1} sodium phosphate buffer pH 7.5, 6.3 mmol L^{-1} EDTA, 0.21 mmol L^{-1} NADPH, 0.6 mmol L^{-1} DTNB and 0.9 U baker's yeast glutathione reductase (205 units mg^{-1} protein;

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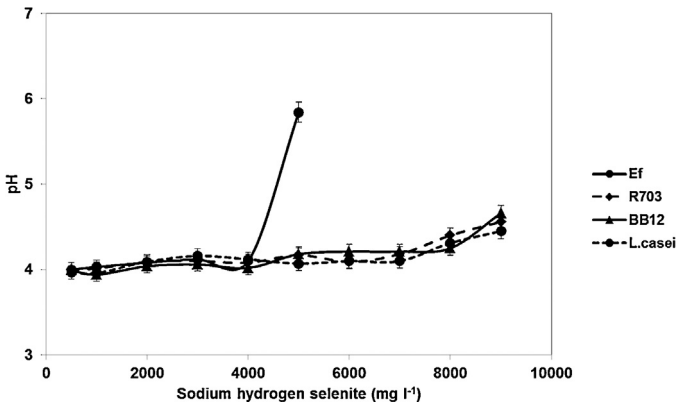

Fig. 2. *Lactobacillus casei* 431 (*L. casei*), *Bifidobacterium animalis* ssp. *lactis* BB12 and *Lactococcus lactis* ssp. *lactis* R703 mutant cultures were more resistant to selenite, while, the metabolism of *Enterococcus faecium* Ef mutant strain collapsed when the cells were exposed to 5000 mg L^{-1} or higher $NaHSeO_3$ concentrations. All measurements were carried out in triplicates. The standard deviations of the mean values were always below 0.35 pH unit and, therefore, they are not presented here for clarity.

G3664; Sigma-Aldrich, Hungary) in a final reaction volume of 1 mL. Quantitation was made using a calibration curve covering the range of 0–5 nmol GSH. The rate of DTNB reduction was monitored spectrophotometrically at λ = 412 nm [15].

For GR activity measurements, cells were disintegrated by sonication in 20 mmol L^{-1} TEA-HCl, pH 7.6, also containing 0.1 mmol L^{-1} EDTA. In a final volume of 1 mL, assay mixtures always contained 50 mmol L^{-1} K_2HPO_4, 0.1 mmol L^{-1} EDTA, pH 7.5, 100 μmol L^{-1} NADPH, 1 mmol L^{-1} GSSG and the cell extract. Enzyme assays were performed at 25 °C, and the decrease in the NADPH was determined at λ = 340 nm. GR activities were determined using a calibration curve in the activity range of 0–0.45 U (GR was from baker's yeast; 205 U mg^{-1} protein; G3664; Sigma-Aldrich, Hungary).

Protein concentrations in bacterial cell extracts were determined using the bicinchoninic acid (BCA Protein Assay Reagent, Thermo Scientific Pierce) reagent with bovine serum albumin (BSA) calibration according to the protocol of the manufacturer. All experiments were repeated three times.

Statistics

Statistical significance was calculated in Student *t*-test and $p \leq 0.05$ was regarded as statistically significant difference between datasets.

Results

Mutants of *E. faecium* W54, *L. lactis* ssp. *lactis* R703, *B. animalis* ssp. *lactis* BB12 and *L. casei* 431 that were resistant to high selenite concentrations were selected in cultivation up to 10,000 mg L^{-1} $NaHSeO_3$ (66.24 mmol L^{-1} Se). At a concentration above 5000 mg L^{-1} $NaHSeO_3$, no outgrowth of *E. faecium* was observed; while considerable higher selenite tolerance was observed with R703, BB12 and *L. casei* 431 strains, because the metabolism (acid production) of the strains was not inhibited up to 9000 mg L^{-1} $NaHSeO_3$ concentration (Fig. 2). However, the selenium content of the cells did not increase significantly ($p \leq 0.05$) above 1000 mg L^{-1} $NaHSeO_3$ treatment, therefore, further investigations were done below that concentration.

The stress tolerance of the mutant strains was different from that of the parental cultures. Especially the changes in the thermo tolerance were obvious as the optimal growth of the mutant strains was pushed to higher temperatures comparing to the parental strains. In *L. casei* 431 mutant strain, the salt and pH resistance were also important (Table 1).

Table 1
Differential characterization of the parental and the selenite tolerant mutant bacteria. The bacterial growth was tested at different temperatures, with 6.5% NaCl or at different pH values in MRS medium after 48 h cultivation time.

Growth conditions						
pH	pH 4.4	pH 6.8	pH 6.8	pH 9.6	pH 6.8	pH 6.8
Temperature	30 °C	30 °C	30 °C	30 °C	10 °C	45 °C
Supplement			NaCl			
Strains						
L. casei†	+‡	+	–	–	±	–
mutant *L. casei*	+	+	±	+	–	–
R703†	+	+	–	+	±	–
mutant R703	+	+	–	+	–	±
BB12†	+	+	±	+	±	–
mutant BB12	+	+	±	±	–	±
E. faecium†	±	±	+	+	–	±
mutant *E. faecium*	±	+	+	+	+	+

† *L. casei*, *Lactobacillus casei* 431; R703, *Lactococcus lactis* subsp. lactis; BB12, *Bifidobacterium animalis* ssp. lactis; *E. faecium*, *Enterococcus faecium*.
‡ +, Growth in 24 h; –, no sign of growth up to 48 h cultivation; ±, weak growth.

Investigating at different pHs (5.97, 6.79, 8.01 and 8.96) and temperatures (28, 32 or 37 °C) R703 and BB12 mutants possessed wider thermo- and pH tolerances than *L. casei* 431 or the *E. faecium* mutant strains as they showed similar growths at all tested temperatures and pH values (Fig. 3A). *L. casei* 431 showed decreased growth at alkaline pHs at 28 °C and 32 °C while the differences in the pH tolerance diminished when it was incubated at 37 °C. Under selenite stress (100 mg L^{-1} and 200 mg L^{-1} $NaHSeO_3$) the pH changes of the medium became more important for the bacterial growth especially for *L. casei* mutant strain (Fig. 3B and C).

The stress tolerance of the *E. faecium* mutant strain was rather versatile. Increasing the SeO_3^{2-} concentration, the optimal growth temperature range became wider; meanwhile, without the stress agent the mutant strain was sensitive to the pH changes and showed narrow temperature tolerance, *e.g.* did not grow properly at 28 °C and 37 °C (Fig. 3B and C). The 32 °C incubation temperature was applicable for all strains on the basis of our results; therefore, all strains were cultivated on this temperature at pH 8.01 for *E. faecium* or at pH 6.79 for further experiments.

The bioaccumulation of selenium was measured in the selenite tolerant mutant strains applying 600 mg L^{-1} $NaHSeO_3$. Dark red pigmentation of the cells was observed in all cultures, especially when the mutant R703 or the mutant *L. casei* strains were cultivated with ~~selenium~~. The biomass production was high (above 400 mg L^{-1}) for all mutant strains except *L. casei* (below 100 mg L^{-1}) after 24 h cultivation (Fig. 4). Remarkable high selenium contents were measured in the lyophilized biomasses (above 40,000 mg Se kg^{-1} dry biomass) and significantly ($p \leq 0.05$) less selenium were accumulated in the mutant *L. casei* strain (Fig. 4).

Intracellular GSH contents and GR activities also were measured in parental and mutant strains of the bacteria. In all mutant strains increased GSH concentrations and GR activities were calculated comparing to that of the parental strains (Table 2). Interestingly, the parental and the mutant *E. faecium* strain possessed exceptionally increased total GSH content and GR specific activity (Table 2) in contrast to the other strains. Under selenite stress, both the GSH content and the GR activities decreased in all strains with the exception of *L. casei*, where both values increased. In the MRS culture medium extracellular GSH content (3.437 μmol mL^{-1} GSH) was detected at the inoculation time and increased concentrations were detected from the culture fluid after 24 h cultivation time, except in *E. faecium* (data not shown).

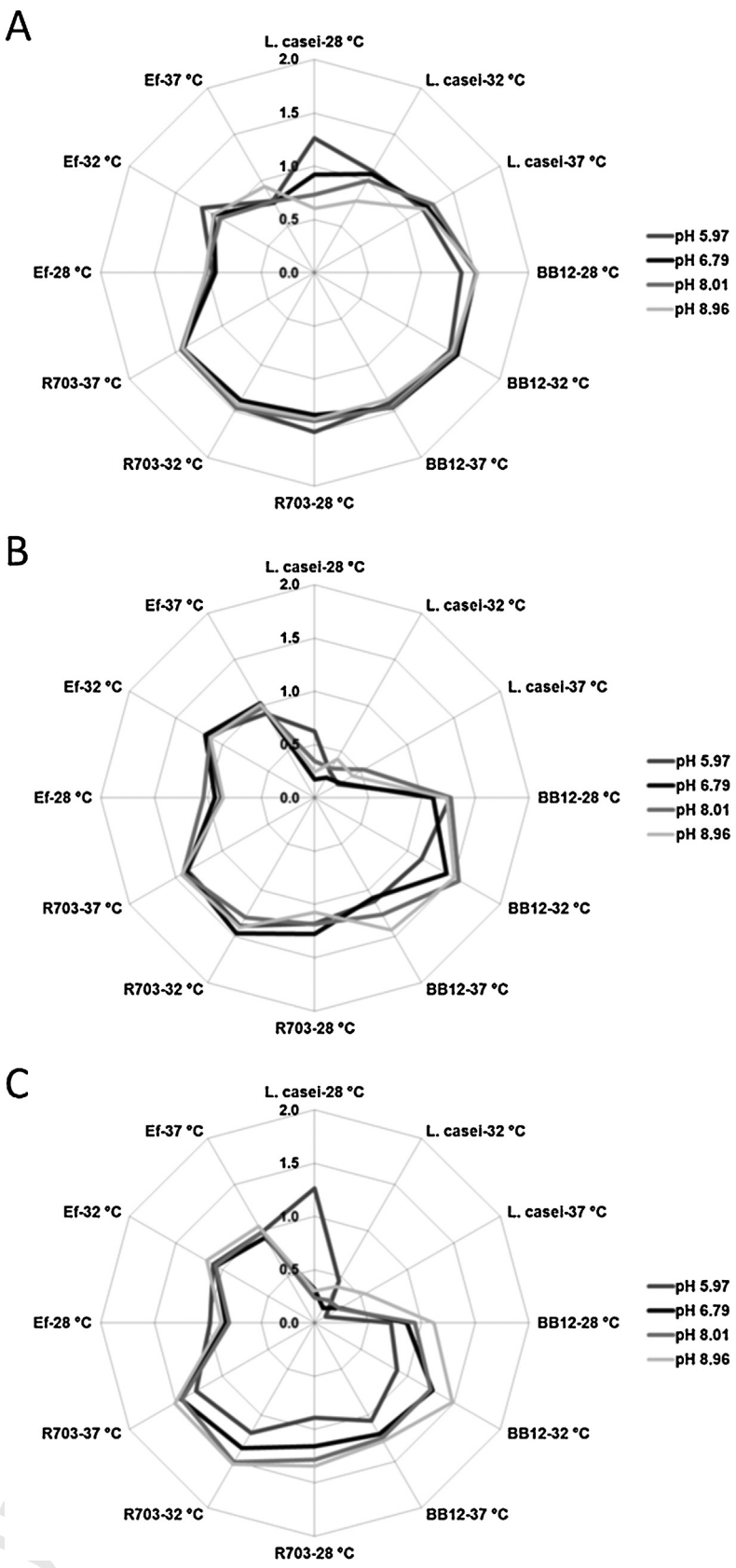

Fig. 3. Growth of the selenite-tolerant mutant strains. Biomass productions were determined at different starting pH values (pH 5.97, 6.79, 8.01 and 8.96) and temperatures (28, 32 or 37 °C). Optical densities (OD_{630} values after 24 h cultivation) were determined spectrophotometrically. The *Lactobacillus casei* 431 (*L. casei*), *Bifidobacterium animalis* ssp. *lactis* BB12, *Lactococcus lactis* ssp. *lactis* R703 and *Enterococcus faecium* (Ef) mutant strains were incubated in MRS medium without aeration. Bacterial growth was recorded without selenite stress (A), in the presence of 100 mg L^{-1} (B) or 200 mg L^{-1} $NaHSeO_3$ (C), respectively. All experiments were carried out in triplicates, and the standard deviations of the mean values were below 10%.

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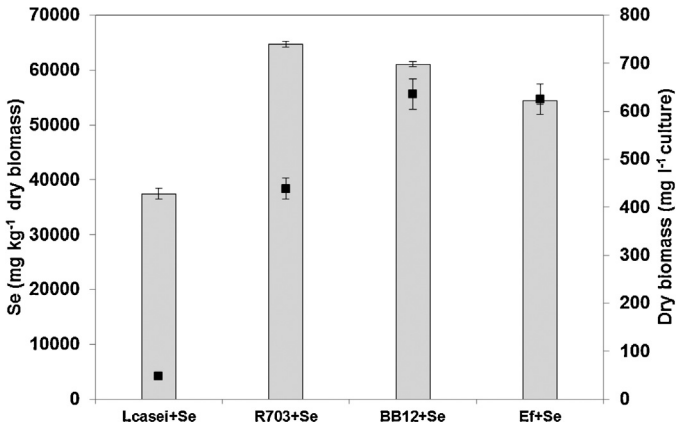

Fig. 4. Biomass production (■) and selenium accumulation (gray bars) of the mutant strains. Cultures of *Lactobacillus casei* 431 (*L.casei*), *Bifidobacterium animalis* ssp. *lactis* BB12, *Lactococcus lactis* ssp. *lactis* R703 and *Enterococcus faecium* (Ef) mutant strains were supplemented with 600 mg L^{-1} $NaHSeO_3$. All experiments were carried out in triplicates; the statistical significance was calculated in Student *t*-test ($p \leq 0.05$). All selenium concentration was significantly different from each other, while the dry biomass of BB12 and Ef were not.

Discussion

Bacterial cells usually possess low tolerance against selenite stress and show limited capability to reduce high concentrations of selenite to elementary selenium. Selenite stress elicits adaptive response in lactic acid bacteria [16–18], therefore, repetitive selection of selenite tolerant *L. casei* 431, *B. animalis* ssp. *lactis* BB12, *L. lactis* ssp. *lactis* R703 and *Enterobacter faecium* W54 strains under increasing selenite stress resulted in promising candidate microbes for future industrial applications.

Optimization of the growth of the selenite tolerant strains revealed significant differences in their thermo tolerance and pH tolerance, which may influence any further utilization of the microbes. Lactococci and lactobacilli are known to have low tolerance against alkaline conditions; however, the R703 and BB12 mutant strains showed remarkable growths in the pH range of 4.4–9.6. Interestingly, Shah [19] reported an optimum growth for *Bifidobacterium* at pH 6–7; meanwhile, no growth was observed above pH 8–8.5, which contrasted strikingly to our results. Pieniz et al. [8] observed growth and selenium bioaccumulation in *E. faecium* cultures between 25 and 45 °C; while, under selenite stress the growth of the *E. faecium* mutant strain decreased considerably below 32 °C. It was also found that the addition of 15 mg L^{-1} Na_2SeO_3 (0.086 mmol L^{-1} Se) to *E. faecium* cultures increased the elementary selenium content of the biomass up to 475.9 ± 19.8 mg kg^{-1} dry biomass [8]. Importantly, significantly higher quantities of selenium (54,362 ± 594 mg kg^{-1} dry biomass) were measured in our mutated *E. faecium* strain when exposed to 3.97 mmol L^{-1} Se (600 mg L^{-1} $NaHSeO_3$) under alkaline pH.

L. casei ssp. *casei* was reported to accumulate 400 mg Se kg^{-1} dry biomass, and at least 80% of it was associated with organic molecules like selenocysteine [20]. However, the *L. casei* 431 strain was also described as a potential nanoselenium producer probiotic organism, which produced 1500–3500 mg Se kg^{-1} dry biomass in the presence of 200 mg L^{-1} $NaHSeO_3$ (1.32 mmol L^{-1} Se) at 37 °C [21]. In our experiments, the mutant of *L. casei* 431 strain accumulated about 10 times more total selenium (37,428 ± 978 mg kg^{-1} dry biomass). While, R703 mutant strain was characterized with wider thermo- and pH tolerances and with higher biomass yields accumulated selenium at a higher level (64,726 ± 545 mg kg^{-1} dry biomass).

GSH has long been suspected to be involved in selenium metabolism, and while there is no detailed investigation in relation of selenite reduction in Gram positive bacteria, there is a detailed work that outlined the processes in Gram negative organisms [10]. There, biotic and abiotic nanoselenium production takes place simultaneously. The main proposed process is the abiotic reduction of selenite that needs high GSH concentration and selenodiglutathione (GS-Se-SG) is the first product of a quick reaction beside superoxide anion (O_2^-). Kessi and Hanselmann [10] concluded that the higher the GSH: selenite ratio (>2), the higher the abiotic nanoselenium production. The next reactions are much slower steps leading to elemental selenium formation. In contrast to these processes biotic reactions need enzymes like glutathione reductase (GR), oxidoreductases and ROS eliminating enzymes like NADH peroxidases. GR reduce GSSG to GSH while oxidizing NADPH to $NADP^+$, which is reduced by oxidoreductases [11].

Only a few lactic acid bacteria and other Gram positive prokaryotes were proven to possess glutathione biosynthetic pathway and most of the lactic acid bacteria were shown to import GSH from their environment [12]. For lactococci, the available data on GSH synthesis were controversy because numerous lactococci were described not to have any capability to perform *de novo* GSH synthesis on minimal medium; while, for some of the lactococci, GSH synthesis was proposed when their genomes were annotated and functionally analyzed [12]. A GSH biosynthetic fusion protein is coded in the genome of *L. casei* (GI:301066250); however, the activity of the gene product has not been demonstrated yet [12]. Moreover, GSH is synthesized by a fusion protein (GshF or GshAB; [22]) in *E. faecium* when grown in MRS or in other complex media, and this could explain the high GSH content of the parental and mutant *E. faecium* strains. Meanwhile, GSH was under the detection level in the other parental strains, GSH was detected in all studied mutant strains meaning a possible elevated GSH uptake. The selenite treatment increased further GR and GSH values in *L. casei* mutant strain pointing at a different selenite reduction pathway and/or stress response in this organism. Glutathione uptake could activate a GSH – glutathione peroxidase (GPx) – GR system, which catalyze the reduction of H_2O_2 (*e.g.* [23]) and GSH protected the cells against acid stress [24] or heat stress and prevented peroxidation of membrane fatty acids [25]. Similarly, a complete glutathione system against oxidative stress was demonstrated in *Lactobacillus fermentum* [26]. Selenite resistance induced stress response systems that lead up-regulation of GR production In the Gram-positive bacterium *E. faecalis*, GR has been purified to homogeneity [27]; meanwhile, no GR activity has been described in *E. faecium* until now. Here, GR activities were detected in *E. faecium* W54 strain but

Table 2
Total glutathione (GSH) and glutathione reductase activity in parental and selenite tolerant mutant bacteria. Supplementation with 600 mg L^{-1} $NaHSeO_3$ was done at the inoculation time.

	Parental	Mutant	Mutant + Se	Parental	Mutant	Mutant + Se
	GSH (μmol GSH mg^{-1} protein)			GR (U mg^{-1} protein)		
L. casei	0	0.019 ± 0.005	0.208 ± 0.012	0.100 ± 0.011	7.938 ± 0.102	23.692 ± 0.542
R703	0	0.369 ± 0.012	0.152 ± 0.017	0.659 ± 0.013	9.902 ± 0.211	3.913 ± 0.176
BB12	0	1.227 ± 0.010	0.075 ± 0.007	0.419 ± 0.018	48.968 ± 0.153	7.546 ± 0.347
E. faecium	0.43 ± 0.007	5.022 ± 0.014	1.136 ± 0.021	0.952 ± 0.015	110.305 ± 2.442	33.766 ± 0.821

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this enzyme activity was minute comparing to the activity of the mutant strain.

The selenite supplementation significantly decreased intracellular GSH concentrations and GR activities. On the basis of the proposed reactions assumed by Kessi and Hanselmann on Gram negative bacteria [10], the low starting GSH concentration, the culmination of GSSG and excess in superoxide anion concentration in case of shortage of ROS eliminating enzymes could disorder abiotic reaction and the cellular homeostasis.

Safe bacterial cultures as functional foods or micronutrient supplements always attract considerable interest from both academic and industrial levels. Significant uptake and transformation of selenite to both organic and inorganic forms of selenium by *E. faecium* and the BB12 mutant strains with concomitantly high biomass production indicated that these strains could be good sources for direct dietary Se delivery [20], *e.g.* in dried form or in dairy products. These bacterial cells, as an addition to the decreased toxicity of selenite to elementary selenium [28,29] possess positive physiological characteristics, which may have further beneficial effects on the consumer's health (*e.g.* antimutagenic activity [30]). The another downstream products of selenium supplementation, selenoproteins (*e.g.* glutathione peroxidase) containing oxidative selenocysteine. Moreover, high GSH accumulation and/or synthesis means greater stability of the strains under storage conditions and quicker growth upon inoculation. Probiotic cells with high antioxidant capacity without selenium fortification also can be used in medicine to modulate antioxidant status, pro-/anti-apoptotic proteins, caspases, and DNA damage [31].

We concluded that our strains are suitable for selenium enriched biomass production can be recommended as food or feed supplement with beneficial effects after further investigation.

Conflict of interest

The authors state that there is no conflict of interest.

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