

Effect of sodium selenite on the bacteria growth, selenium accumulation, and selenium biotransformation in *Pediococcus acidilactici*

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Abstract In this study, the effect of low selenium concentrations on bacteria growth, selenium bioaccumulation, and selenium speciation in *Pediococcus acidilactici* was investigated. Six different sodium selenite (Na_2SeO_3) solutions with concentrations of 0, 0.5, 1, 2, 3, and 4 mg/L were added in MRS broth for 24 h. Then, the obtained bacterial pellets were weighed. The contents of total selenium and selenium species in the bacterial pellets were measured via optimized enzymatic hydrolysis and HPLC-ICP-MS. The maximum dried *P. acidilactici* biomass of 1.44 g/L was achieved by utilizing 1 mg/L Na_2SeO_3 . By increasing sodium selenite concentrations, total selenium contents were significantly increased from 0.14 to 1.45 mg/g dry weight ($p < 0.05$). The findings indicated that selenium was favorably incorporated into the bacteria protein fraction and mainly formed selenocysteine. Therefore, selenium-enriched lactic acid bacterium *P. acidilactici* can deliver a less-toxic, more bioavailable selenium source for human and animal nutrition.

Keywords Selenium (Se) · Sodium selenite (Na_2SeO_3) · Lactic acid bacteria (LAB) · *Pediococcus acidilactici* · Enrichment

Introduction

Lactic acid bacteria (LAB) can absorb metal and metalloid ions into their cell surface via numerous functional groups or inside the cell via several procedures depending on the chemical features of the microelement, physiological characteristics of strain, and physicochemical properties of environment [1]. Their applications include the removal of highly toxic elements from foods and creation of nutraceuticals [2]. LAB also have notable health-promoting features including anticarcinogenic actions, hypocholesterolemic characteristics, and antagonistic potentials against gastrointestinal pathogens [3].

The benefits of LAB can significantly be improved by increasing the essential elements and total amino acids in the presence of selenite in the medium [4]. Biotransformations of selenite through LAB have been detailed [5–7], mainly being selenocysteine and selenomethionine. Such biotransformations deliver a less-toxic, more-bioavailable selenium source for human and animal nutrition while greatly enhancing the inhibitory effect of the LAB on the growth of the pathogenic bacteria. For example, Se-enriched *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus* indicatively decrease the amount of *E. coli* [8]. The protective effect of selenium was indicated against the toxic amounts of cadmium in *Lactobacillus casei rhamnosus* was obtained from the commercial product of fermented milk [9]. However, the effects of selenite on LAB have been evaluated in some finite species.

Pediococcus acidilactici, a species of gram-positive cocci of the family Lactobacillaceae, can cultivate in a wide range of pH, temperature, and osmotic pressure and can hence greatly colonize the digestive tract [10]. It has emerged as a promising probiotic in human and animal

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nutrition [3, 11–13]. *P. acidilactici* can be favorably used in fermented vegetables, dairy products, and meat, whereas commercially available probiotics mainly belong to the genera of *Lactobacillus* and *Bifidobacterium* [14, 15]. Most of their strains are sensitive to room temperature and acidic exposure, resulting in concerns regarding the desirable storage conditions and subsequently the quality of fermented products [16]. Achieving persistent and reproducible outcomes becomes a major challenge in commercial-functional-food production. Since selenium biotransformation mostly occurs in the bacterial cells when the low sodium selenite levels have been taken by the bacteria, the aim of this study is to evaluate the influence of different low concentrations of sodium selenite on the bacteria growth, selenium accumulation, and selenium biotransformation in *P. acidilactici*.

Materials and methods

Chemicals

All chemicals were of analytical grade and all solutions were prepared with deionized water (Merck Millipore, Germany). Stock solutions were prepared, kept at 4 °C, and daily diluted to provide working and standard solutions. Sodium selenite (Na_2SO_3) was obtained from Merck, Germany. Selenomethionine (SeMet), selenocysteine (SeCys₂), and Se-methylselenocysteine (SeMeSeCys) were purchased from Sigma-Aldrich, Germany. Bacterial pellets were extracted using hydrolytic enzymes namely, lysozyme, lipase, and protease XIV were obtained from Sigma-Aldrich in a buffer solution containing a mixture of Tris base, acetic acid, and EDTA (TAE) [5].

Instruments

A microwave digestion set (MW500, Merck, Germany) was used for digestion of the bacterial samples. An ultrasonic processor (UP400S, Hielscher, Germany) with a 3-mm diameter titanium sonotrode fitted with a frequency generator of 400 W at 24 kHz was used to extract the seleno-aminoacids from the bacterial pellet. A Perkin Elmer Elan 6100 DRC-e inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer, Inc., Waltham, MA, USA) was applied for measuring the total selenium and selenium species under ICP-MS operating conditions including radio frequency (RF) power, plasma gas (argon) flow rate, nebulizer gas flow rate, and sample uptake rate that were respectively maintained at 1250 W, 15.0 l/min, 0.84 l/min, and 1.5 ml/min. The isotopes of ⁷⁶Se, ⁷⁷Se, ⁷⁸Se, and ⁸²Se were monitored. Separation of inorganic and organic selenium was conducted using HPLC (Knauer,

Berlin, Germany). The anion exchange column PRP X100 with the dimensions 4.1 mm × 250 mm and particle size 10 μm (Hamilton, Switzerland) was used for chromatography. Selenium species separation was realized by 10 mM ammonium citrate (Merck, Germany) in 2% methanol (Hangzhou ICH Biopharm, China) adjusted to pH 5.5 using diluted ammonium hydroxide (Merck, Germany) in the mobile phases. The sample flow rate was 1.5 ml/min.

Bacteria culture

Pediococcus acidilactici (ATCC[®] 8042[™]) was obtained from ATCC, Manassas, USA. Freeze-dried bacterial stock was kept at 4 °C. The bacterial stock was rehydrated as manufacturer's instructions. The bacteria were cultured in MRS broth (Merck KGaA, Germany) prepared according to the manufacturer's instructions. Similar suspension volumes of 1%(v/v) were used for inoculation into the MRS medium at 37 °C for 24 h [17].

Preparation of the selenium-enriched bacteria

After initial growth for 24 h, the cultures were exposed to different sodium selenite concentrations, 0, 0.5, 1, 2, 3, and 4 mg/L, for an additional 24 h. Then, bacterial cells were centrifuged at 3000×g for 10 min and washed twice with deionized water. The obtained bacterial pellets were lyophilized and weighed.

Total selenium measurement using ICP-MS

The bacterial pellets were digested with 1 mL HNO_3 (Merck Millipore, Germany) and H_2O_2 (Carlo Erba, France) using the microwave oven [5]. After cooling, the digests were diluted to 20 ml with deionized water, and selenium concentration of the solutions was determined using ICP-MS.

Selenium species measurement using HPLC-ICP-MS

The amount of selenium species in the bacterial pellets were determined using enzymatic hydrolysis and HPLC-ICP-MS. An optimized enzymatic procedure was applied to enhance selenium species recovery in the bacterial pellets. First, 0.5 mL of 0.1 g/L lysozyme (pH 7.5) was added to 0.05 mg of the bacterial pellet for 2 h at 37 °C. Then, 0.5 mL solution of 0.1 g/L protease XIV (pH 7.5) was added and mixed using an ultrasonic processor (Hielscher, Germany) with a 3-mm diameter titanium sonotrode for 2 min. The extracts from the bacterial pellets were centrifuged at 3000×g for 10 min using a 0.2 nm cutoff filter and were kept at −80 °C for further analysis [5]. To investigate the effect of the enzymatic hydrolysis on

selenium recovery from the bacterial pellets, the selenium concentration in the obtained extracts was measured via ICP-MS. Furthermore, selenium species in the extracts were determined via HPLC-ICP-MS using anion exchange chromatography. All experiments were conducted in triplicate.

Statistical analysis

Data analysis was performed via a multiple-dominant Duncan test utilizing IBM SPSS Statistics 23 (IBM corporation, New York, USA). A P value of less than 0.05 was assigned statistically significant. Data were expressed as mean \pm standard deviation (SD).

Results and discussion

Selenium-enriched *P. acidilactici* growth

Selenium is an essential element for different living organisms, from bacteria to humans, because of its unique role in the structure of selenium-containing macromolecules [18]. To investigate the effect of selenium on the growth of *P. acidilactici*, different concentrations of sodium selenite between 0 and 4 mg/L were added in the MRS medium. As can be seen from Fig. 1, selenite did not inhibit the bacteria growth and *P. acidilactici* biomass significantly increased for the 1 and 2 mg/L Na_2SeO_3 ($p < 0.05$). *P. acidilactici* biomass was first increased by increasing the sodium selenite concentration from 0 to 1 mg/L, then gradually decreased, and eventually nearly plateaued. The maximum dried *P. acidilactici* biomass of 1.44 g/L was obtained with 1 mg/L sodium selenite.

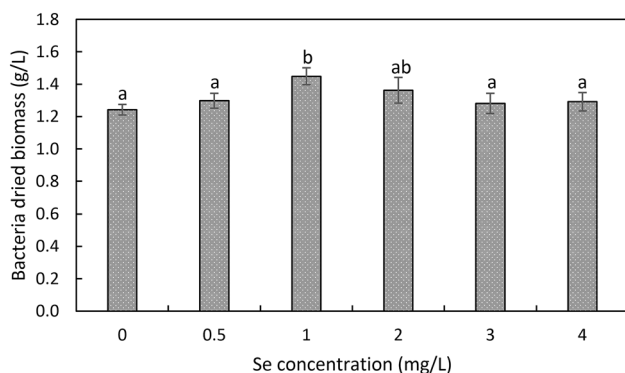


Fig. 1 Mean dried biomass of *Pedicoccus acidilactici* grown under different sodium selenite concentrations ($T = 37 \pm 1$ °C, $\text{pH} = 6.5 \pm 0.2$, Time = 24 h). Means marked with different letters are significantly different ($p < 0.05$)

This observation can be attributed to the fact that the selenium accumulation in LAB indicates a biphasic trend in the formation of total amino acids [4]. The low sodium selenite levels significantly increase the total amino acids in the bacterial cells, whereas the high concentrations of selenium activate detoxification processes, which transform selenite to elemental selenium (Se^0), which is deposited near of bacterial membranes into cells [1, 4, 19]. Moreover, Se^0 deposits noticeably accumulate near the periphery of the bacterial cells in the presence of high sodium selenite levels [1, 4]. Hereby, using the suitable amount of selenium in the culture medium may increase the amount of total amino acids and consequently bacteria growth. The obtained results were in agreement with the literature where growth of *Lactobacillus rhamnosus*, isolated from goat milk, were significantly increased in the presence of 2 mg/L Se (IV) in the MRS medium [20]. Moreover, the highest growth of the Se-enriched bacterial strains *Lactobacillus casei* and *Lactobacillus paracasei* was achieved by utilizing 1.0–2.5 mg/L Na_2SeO_3 [21].

Selenium extraction

The used extraction method strongly influences the quantity of selenium obtained from the bacterial cells [5]. Therefore, an optimized extraction method was required to recover the maximum amount of selenium species from the bacterial pellets. Among different extraction procedures, enzymatic hydrolysis is more efficient than aqueous extraction and acid hydrolysis for selenium recovery [6, 21]. Therefore, the effect of five different enzymatic hydrolyses, lysozyme, lipase, protease XIV, a combination of lipase and protease XIV, and lysozyme and protease XIV, on selenium extraction was studied. The maximum selenium recovery of 98.5% was realized using a mixture of lysozyme and protease XIV, while the lowest selenium recovery of 14.9% was obtained using lipase alone, as depicted in Table 1. The findings show that higher selenium recovery was realized using protease XIV than using lipase and lysozyme, and the selenium recovery yield was greatly affected by the previous step (Table 1).

The better selenium recovery obtained using protease XIV were possibly due to this being a mixture of at least three proteolytic active substances such as extracellular serine protease resulted in greater hydrolysis of peptide bonds [22]. Moreover, it appeared that disruption of the bacterial cell wall probably is more efficient than bacterial cell-membrane destruction for selenium recovery. Lysozyme-enhanced seleno-compound recovery via damaging bacterial cell walls through the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-

Table 1 Enzymatic hydrolysis optimization used for selenium extraction from *P. acidilactici* cell pellet (Se concentration = 1 mg/L, T = 37 ± 1 °C, pH = 6.5 ± 0.2, Time = 24 h)

Enzymatic hydrolysis	Extracted Se (µg)	Total Se (µg)	Extraction efficiency (%)
Lysozyme	79.3 ^a ± 4.8	155.7 ± 9.7	50.9
Lipase	22.7 ^b ± 1.6		14.6
Protease	113.8 ^c ± 12.5		73.1
Lipase + Protease	127.5 ^c ± 11.5		81.9
Lysozyme + Protease	153.4 ^d ± 13.8		98.5

Data are presented as mean ± SD (n = 3)

Means with different letters are significantly different ($p < 0.05$)

glucosamine residues in a peptidoglycan layer of most bacterial extracellular cell wall, whereas lipases often cut a specific position on the glycerol backbone of a lipid substrate of the prokaryote cell membrane [23]. The obtained results were in agreement with the literature, where selenium-recovery efficiencies realized using protease XIV were 91.5 and 92% in 1 and 2 µg/g Se (VI) [5].

Selenium bioaccumulation

LAB accumulate selenium into the cell through different processes depending on the characteristics of the bacterial species and experimental environment. By increasing sodium selenite concentrations in the *P. acidilactici* medium from 0.5 to 4 mg/L, total selenium contents significantly increased from 0.14 to 1.45 mg/g dry weight ($p < 0.05$), indicating that *P. acidilactici* could feasibly accumulate selenium into the biomass (Table 2). This may be attributed to the fact that LAB can uptake inorganic selenium from the medium, transform it into its organic forms, and assimilate it into different cellular fractions, e.g., protein, polysaccharide, and nucleic acid components [2, 19]. These findings are in agreement with those of previous works where LAB *L. delbrueckii*, *L. plantarum*, and *L. casei* could respectively accumulate 0.25, 0.38 and 0.41 mg/g Se (IV) in the presence of 1 mg/L Na₂SeO₃ [24].

Selenium biotransformation by *P. acidilactici*

Selenium is mainly assimilated into LAB proteins as selenocysteine and selenomethionine [5, 19]. *P. acidilactici* was exposed to different sodium selenite concentrations, 0, 0.5, 1, 2, 3, and 4 mg/L in the MRS medium to determine the different seleno-amino acids via HPLC-ICP-MS. As shown in Table 2, no seleno-amino acids were determined in *P. acidilactici* in the control treatment (0 mg/L Na₂SeO₃), whereas the concentrations of organic selenium including selenocysteine, methylselenocysteine, and selenomethionine in the bacterial pellets increased by increasing the sodium selenite concentrations from 0.5 to 4 mg/L (Fig. 2).

Selenium substitutes sulfur in cysteine and methionine in the bacterial proteins to form selenocysteine and selenomethionine [25]. The obtained organic selenium was mainly found in the catalytic site of several known redox-active enzymes as non-covalently attached cofactors [18]. Alzate et al. [6] demonstrated that lactic fermentation of yogurt resulted in increased amounts of selenocystine and methylselenocysteine, but kefir production via fermentation increased the selenomethionine concentration. Moreover, yogurt fermentation in the presence of up to 2 µg/g Se (IV) demonstrated an integration of selenium into proteins where selenium species measurement via HPLC-ICP-MS demonstrated the presence of Se-cysteine [5]. Therefore, selenocysteine might be the main seleno-amino acid in the protein fraction of Se-enriched *P. acidilactici*.

Table 2 Total selenium, selenocysteine, methylselenocysteine, and selenomethionine concentrations in *P. acidilactici* grown under different sodium selenite levels in MRS broth (T = 37 ± 1 °C, pH = 6.5 ± 0.2, Time = 24 h)

Na ₂ SeO ₃ concentration (mg/L)	Total Se (mg/g)	SeCys ₂ (µg/g)	MeSeCys (µg/g)	SeMet (µg/g)
0	ND ^a	ND ^a	ND ^a	ND ^a
0.5	0.17 ^b ± 0.01	86.75 ^b ± 7.70	15.10 ^b ± 2.60	10.74 ^b ± 1.27
1	0.43 ^c ± 0.04	201.52 ^c ± 19.06	70.29 ^c ± 5.33	31.43 ^c ± 3.61
2	0.87 ^d ± 0.05	419.04 ^d ± 33.61	121.60 ^d ± 11.35	114.11 ^d ± 6.33
3	1.36 ^e ± 0.12	539.63 ^e ± 50.21	205.61 ^e ± 18.59	123.82 ^d ± 5.88
4	1.89 ^f ± 0.20	809.24 ^f ± 67.31	252.22 ^f ± 22.67	195.76 ^e ± 15.23

^a Not detected

Data are presented as mean ± SD (n = 3)

Means with different letters are significantly different ($p < 0.05$)

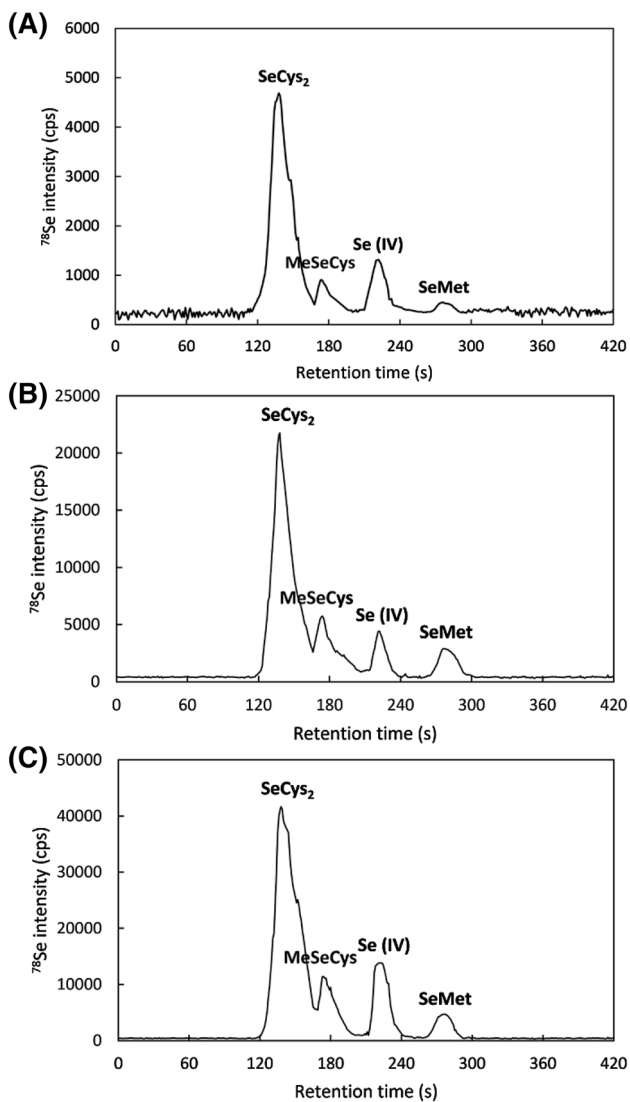


Fig. 2 Anion-exchange HPLC-ICP-MS chromatograms of hydrolytic extracts of *P. acidilactici* cell pellets grown under different sodium selenite (Na_2SeO_3) levels: (A) 0.5 mg/L, (B) 2 mg/L, and (C) 4 mg/L in MRS broth ($T = 37 \pm 1^\circ\text{C}$, $\text{pH} = 6.5 \pm 0.2$, Time = 24 h)

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Mrvčić J, Stanzer D, Šolić E, Stehlik-Tomas V. Interaction of lactic acid bacteria with metal ions: opportunities for improving food safety and quality. *World J. Microbiol. Biotechnol.* 28: 2771–2782 (2012)
- Pophaly SD, Singh P, Kumar H, Tomar SK, Singh R. Selenium enrichment of lactic acid bacteria and bifidobacteria: A functional food perspective. *Trends Food Sci. Technol.* 39: 135–145 (2014)
- Balcázar JL, De Blas I, Ruiz-Zarzuola I, Cunningham D, Vendrell D, Múzquiz JL. The role of probiotics in aquaculture. *Vet. Microbiol.* 114: 173–186 (2006)
- Xia SK, Chen L, Liang JQ. Enriched selenium and its effects on growth and biochemical composition in *Lactobacillus bulgaricus*. *J. Agr. Food Chem.* 55: 2413–2417 (2007)
- Alzate A, Cañas B, Pérez-Munguía S, Hernández-Mendoza H, Pérez-Conde C, Gutiérrez AM, Cámara C. Evaluation of the inorganic selenium biotransformation in selenium-enriched yogurt by HPLC-ICP-MS. *J. Agr. Food Chem.* 55: 9776–9783 (2007)
- Alzate A, Fernández-Fernández A, Perez-Conde M, Gutiérrez A, Cámara C. Comparison of biotransformation of inorganic selenium by *Lactobacillus* and *Saccharomyces* in lactic fermentation process of yogurt and kefir. *J. Agr. Food Chem.* 56: 8728–8736 (2008)
- Palomo M, Gutiérrez AM, Pérez-Conde MC, Cámara C, Madrid Y. Se metallomics during lactic fermentation of Se-enriched yogurt. *Food Chem.* 164: 371–379 (2014)
- Yang J, Huang K, Qin S, Wu X, Zhao Z, Chen F. Antibacterial action of selenium-enriched probiotics against pathogenic *Escherichia coli*. *Digest. Dis. Sci.* 54: 246–254 (2009)
- Araúz ILC, Afton S, Wrobel K, Caruso JA, Corona JFG, Wrobel K. Study on the protective role of selenium against cadmium toxicity in lactic acid bacteria: An advanced application of ICP-MS. *J. Hazard. Mater.* 153: 1157–1164 (2008)
- Biswas S, Ray P, Johnson M, Ray B. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. *App. Env. Microbiol.* 57: 1265–1267 (1991)
- Burr G, Gatlin D, Ricke S. Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. *J. World Aquacult. Soc.* 36: 425–436 (2005)
- Fernandez B, Savard P, Fliss I. Survival and metabolic activity of pediocin producer *Pediococcus acidilactici* UL5: Its Impact on intestinal microbiota and *Listeria monocytogenes* in a model of the human terminal ileum. *Microbiol. Ecol.* 72: 931–942 (2016)
- Gatesoupe F-J. Probiotic and formaldehyde treatments of *Artemia nauplii* as food for larval pollack, *Pollachius pollachius*. *Aquaculture* 212: 347–360 (2002)
- Attri P, Jodha D, Gandhi D, Chanalia P, Dhanda S. In vitro evaluation of *Pediococcus acidilactici* NCDC 252 for its probiotic attributes. *Int. J. Dairy Technol.* 68: 533–542 (2015)
- Barros RR, Maria Da Glória SC, Peralta JM, Facklam RR, Teixeira LM. Phenotypic and genotypic characterization of *Pediococcus* strains isolated from human clinical sources. *J. Clin. Microbiol.* 39: 1241–1246 (2001)
- Mizutani W, Yamasaki R, Lin JJ, Kuki M, Kato G. *Pediococcus* a unique probiotic we use as a novel GI supplement. *Ann. Meet. Jpn. Brd. Vet. Pract.*: 3269–3272 (2007)
- Altuntas EG, Cosansu S, Ayhan K. Some growth parameters and antimicrobial activity of a bacteriocin-producing strain *Pediococcus acidilactici* 13. *Int. J. Food Microbiol.* 141: 28–31 (2010)
- Stolz JF, Basu P, Santini JM, Oremland RS. Arsenic and selenium in microbial metabolism. *Ann. Rev. Microbiol.* 60: 107–130 (2006)
- Zhang B, Zhou K, Zhang J, Chen Q, Liu G, Shang N, Qin W, Li P, Lin F. Accumulation and species distribution of selenium in Se-enriched bacterial cells of the *Bifidobacterium animalis* 01. *Food Chem.* 115: 727–734 (2009)
- Andreoni V, Luischi MM, Cavalca L, Erba D, Ciappellano S. Selenium tolerance and accumulation in the *Lactobacillus* species. *Ann. Microbiol.* 50: 77–88 (2000)
- Kurek E, Ruszczynska A, Wojciechowski M, Łuciuk A, Michalska-Kacymirów M, Motyl I, Bulska E. Bio-transformation

- of selenium in Se-enriched bacterial strains of *Lactobacillus casei*. *Rocz. Panstw. Zakl. Hig.* 67: 253–262 (2016)
22. Barwick M, Maher W. Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Env. Res.* 56: 471–502 (2003)
 23. Imoto T, Yagishita K. A simple activity measurement of lysozyme. *Agr. Bio. Chem.* 35: 1154–1156 (1971)
 24. Calomme M, Branden K, Berghe D. Selenium and *Lactobacillus* species. *J. App. Bacteriol.* 79: 331–340 (1995)
 25. Rother M. Selenium metabolism in prokaryotes. pp. 457–470. In: Selenium: its molecular biology and role in human health. Hatfield DL, Berry MJ, Gladyshev VN (eds). Springer, New York, USA (2011)