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Zinc Binding by Lactic Acid Bacteria

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Summary

Zinc is an essential trace element in all organisms. A common method for the prevention of zinc deficiency is pharmacological supplementation, especially in a highly available form of a metalloprotein complex. The potential of different microbes to bind essential and toxic heavy metals has recently been recognized. In this work, biosorption of zinc by lactic acid bacteria (LAB) has been investigated. Specific LAB were assessed for their ability to bind zinc from a water solution. Significant amount of zinc ions was bound, and this binding was found to be LAB species-specific. Differences among the species in binding performance at a concentration range between 10–90 mg/L were evaluated with Langmuir model for biosorption. Binding of zinc was a fast process, strongly influenced by ionic strength, pH, biomass concentration, and temperature. The most effective metal-binding LAB species was *Leuconostoc mesenteroides* (27.10 mg of Zn^{2+} per gram of dry mass bound at pH=5 and 32 °C, during 24 h). FT-IR spectroscopy analysis and electron microscopy demonstrated that passive adsorption and active uptake of the zinc ions were involved.

Key words: zinc, *Lactobacillus*, adsorption, trace elements, LAB

Introduction

Recent publications have shown many pathogenic deviations in humans, animals, and microbial organisms induced by excess, deficiency or improper proportions of microelements in food and feed substrates (*1–3*). At optimal concentrations, microelements enhance the physiological state of organisms and are essential for many biochemical reactions. Also, microelements neutralize electrostatic forces present in various cellular anions, particularly in cell membranes and the DNA double helix. It is well established that some trace elements, because of their involvement in conformation of antioxidant enzymes, may contribute to the prevention of oxidative stress (*4,5*). The results obtained from some experiments have shown that the dietary intake of trace elements can protect cells from the potential damage caused by free radicals. Enzymes superoxide dismutase, catalase and glutathione peroxidase, which include cofactors Se, Zn, Mn and Fe, form a network of functionally overlapping defense mechanisms.

It is well known that zinc is an essential element required by all living organisms. Zinc is essential for normal growth and development, and for most aspects of reproduction. Next to iron, it is the most abundant trace mineral in the body. It is structural constituent of many enzymes and proteins, including metabolic enzymes, transcription factors and cellular signaling proteins (*6*). It plays an important role in the immune system, in the regulation of appetite and stress level. The typical daily intake of zinc in the Western diet is approx. 10 mg, two-thirds of the recommended dietary allowance (RDA) (*4*). In Croatia, the imbalance of trace elements is frequently found, and the analysis of trace elements in hair has shown that the most deficient trace elements are

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Mn, Fe, Se, Zn and Cr in women and Mn, Se, Zn, Fe and Cr in men. Zn is categorized as deficient in about 30 % of participants (*7*). Because of that, zinc has been recognized as an essential dietary supplement in human and animal nutrition, providing beneficial effects on health.

On the other hand, specific microorganisms are able to absorb numerous metal ions (*8–10*), but this principle has mainly been investigated from an environmental point of view (*11*). Because of that, only soil bacteria, waste yeast or marine algae have been investigated. The efficiency of metal uptake by biomass depends on the chemistry of the metal ions, the specific surface properties of the microorganisms, cell physiology and physicochemical influences from the environment. Usually two processes are known: biosorption and bioaccumulation. Biosorption is a passive, non-metabolically mediated process that may include adsorption, ion exchange, complexation, chelation and microprecipitation (*12*). It is often followed by a slower metal binding process, bioaccumulation, in which binding of metal ions with intracellular structures takes place (*13*).

In the present study biosorption of zinc by lactic acid bacteria (LAB) has been investigated. The used LAB are nonpathogenic, food safe microorganisms, which are commonly applied in food processing. LAB enriched with zinc could be a valuable source of this element in food, because zinc organic compounds (metalloproteins or bioplexes) are the best form for absorption by humans. In that form, their absorption rates are higher than in inorganic compounds (*14*). Microelements bound in the form of protein complexes are absorbed in the small intestine in a manner typical of peptides and proteins that enables penetration of microelements in the intestinal wall. Supplementation of zinc-deficient groups with zinc-enriched LAB (or some other microelement) may be a new promising application of LAB in addition to their probiotic activity. It is well known that some species of LAB concentrate high levels of manganese (*15*). Also, the ability of lactic acid bacteria to concentrate selenium, chromium and to bind toxic ions of cadmium and lead was evaluated (*16–21*). On the other hand, principles of zinc binding by *Lactobacillus* species have not been investigated. Because of that, in this work, the influence of different process parameters on zinc binding by different LAB species is investigated.

Materials and Methods

Microorganisms

The following species of LAB were used throughout the study: *Leuconostoc mesenteroides*, *Lactobacillus brevis* and *Lactobacillus plantarum*. They were taken from the culture collection of the Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia, which were identified for this work in BCCM/LMG Bacteria Collection, Gent, Belgium. The stock cultures were stored at 4 °C on MRS agar (Biolife, Italy) and subcultured every month. These species were selected because of their extensive use in food processing.

Preparation of bacterial biomass

Bacteria were grown under microaerobic conditions at 32 °C for 24 h in MRS broth (Biolife, Italy). Bacterial cells were separated from the culture medium by centrifugation (5000 rpm, 20 min) and washed twice with ultrapure water. Bacterial suspension was used in zinc binding experiment. Bacterial biomass concentration (dry mass) was determined gravimetrically: cells were separated from the culture medium by centrifugation (5000 rpm, 10 min) and dried.

Zinc binding experiments

The effect of initial zinc concentration in synthetic solutions on zinc binding was examined for all tested LAB species. The experiments were conducted in 300-mL Erlenmeyer flasks containing 100 mL of zinc synthetic solutions at a concentration of 10 to 90 mg/L. Metal ion solution was prepared by diluting 20 g/L of stock metal ion solution, which was prepared by dissolving a mass quantity of $ZnSO_4 \cdot 7H_2O$ of analytical reagent grade (Merck) in redistilled water. The concentration of bacterial biomass (dry mass) was 0.8 g/L. Negative control contained bacteria in redistilled water and the positive control contained only zinc in redistilled water. The flasks were agitated on a rotary shaker (Certomat, Braun, Germany) at 100 rpm and 32 °C for 24 h. These results indicated that *L. mesenteroides* was the best zinc-binding species. The influence of the pH, incubation time, temperature and concentration of biomass on zinc binding were examined for this LAB species only.

Determination of the effect of pH on zinc binding by LAB was investigated using different initial pH values (pH=3, 4, 5 or 6), the initial zinc concentrations of about 20 mg/L and 0.5 g/L of bacterial biomass, at 32 °C during 24 h. The pH of the solution was adjusted with 1 M HNO_3 and 1 M NaOH solutions, using a pH meter (Orion 720A, Germany).

The effect of incubation time on zinc binding was examined with initial zinc concentration of about 20 mg/L and 0.6 g/L of bacterial biomass at 32 °C during 24 h. Samples were taken from the suspension and centrifuged every 3 h. Supernatant fractions were analyzed for the remaining metal ions.

The effect of incubation temperature on zinc binding was investigated with initial zinc concentration of about 20 mg/L and 0.7 g/L of bacterial biomass. The experiments were conducted at 8, 12, 32 and 37 °C during 24 h.

The influence of bacterial biomass concentration on zinc binding was examined using different concentrations of bacterial biomass and the initial zinc concentration of 50 mg/L. Bacterial biomass concentration varied from 0.5 to 1.7 g/L. The experiments were conducted at 32 °C during 24 h.

Analysis of zinc content

After incubation, samples were taken from the suspension. The samples were centrifuged for 20 min at 5000 rpm. The supernatant fractions were analyzed for the remaining zinc ions. A volume of 1 mL of the su-

pernatant was preserved with 10 mL of 1 M HNO_3 and the residual metal ion concentration was determined by Flame Atomic Absorption Spectrophotometer (Varian, Spectra AA 300, Varian Techtron, Victoria, Aurstralia). Commercial reference whey powder (IAEA-155) was used for quality control. The values of standard deviation were calculated from the data obtained from three separate experiments.

Electron microscopy

Cell suspension was fixed for 30 min in 1.5 % glutaraldehyde, and then the cells were suspended in warm 2 % agar. After cooling, the agar was cut into cubes with a razor blade. Specimens in the cubes were rinsed with 0.05 M cacodylate buffer (pH=7.2) at 4 °C and then postfixed for 1 h with 1 % osmium tetroxide, in the same buffer at 4 °C. The specimens were dehydrated through an ethanol series and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using an FEI Morgagni 268 electron microscope. Binding experiments for electron microscopy were performed at 32 °C, pH=4.5 during 24 h, with Zn^{2+} concentration of 20 mg/L.

FT-IR spectroscopy analysis

The IR spectra of two samples, dried blank biomass of different LAB species and dried biomass of different LAB species exposed to zinc ions at bacterial concentration of 0.8 g/L, at 32 °C and pH=4.5, after 24 h, and about 20 mg/L of initial metal ion concentration were recorded. After incubation, samples were taken and centrifuged at 5000 rpm for 20 min. Bacterial cells were washed twice with ultrapure water and dried in vacuum. The samples for IR analysis were prepared by pressing powered KBr pellets mixed with 1 % of ground powder of each sample and determined on a Bomem MB 100 Mid FT spectrophotometer. The spectra were recorded in the region of 2000–500 cm^{-1} at a resolution of 4 cm^{-1}.

Simulated gastric juice experiments

Simulated gastric juice was prepared according to Kos *et al.* (*22*). It was prepared by suspending pepsin from porcine gastric mucosa (3 g/L) in sterile sodium chloride solution (0.5 %) and adjusting the pH to 2.5 with concentrated HCl. Pepsin was obtained from Sigma-Aldrich, USA. After biosorption, the biomass was centrifuged and exposed to simulated gastric juice for 4 h at 32 °C. Cell viability experiments were performed according to the method also described by Kos *et al.* (*22*).

Statistical analysis

Experiments were performed in triplicate, standard deviations were calculated, error bars represent averages of 3 experiments of three replicates with standard deviation. The statistical data analysis was carried out using data analysis software system Statistica, v. 7.1, Statsoft, Inc, Tulsa, OK, USA.

Results and Discussion

Biosorption capacity of different LAB species

Metal ion accumulation by fungi and yeasts has been thoroughly researched (*9*,*10*,*13*). However, little is known about metal ion uptake by LAB. The ability of LAB to bind trace elements was examined only for antioxidant element selenium (*23*,*24*). Protective effect of selenium-enriched *Lactobacillus* spp. on mice was established by elevating antioxidant enzyme activities and by reducing peroxidation reaction (*25*). Furthermore, the use of selenium-enriched lactobacillus in the preparation of functional food was studied (*16*). On the other hand, the ability and mechanism of zinc binding by LAB has not been defined.

The biosorption process of metal ions by microorganisms depends mainly on the chemistry of the metal ions, external conditions and the microorganism species (*11*). Differences among bacterial species in the magnitude of change of metal ion binding capacity may be due to properties of the bacteria (*e.g.* structure, functional groups and surface area) depending on the bacterial division, genera and species. Because of that, the biosorption of zinc ions by different LAB species was investigated as a function of initial metal ion concentration.

Results presented in Fig. 1 demonstrate that in the test solutions zinc tended to be bound in a LAB-dependent manner, and that the differences among the species were statistically highly significant ($p \leq 0.001$). The biosorption of zinc to *L. plantarum* appeared to be the lowest among the three LAB species, while *L. mesenteroides* showed the highest ability for zinc ion binding. Also, it was obvious that the absorption of zinc was concentration-dependent for all tested species. Lower binding of zinc ions was achieved at a higher initial metal ion concentration. When the initial zinc concentration was increased from 10 to 90 mg/L, the binding of zinc decreased from 70 to 25 % for *L. mesenteroides*, from 60 to 14 % for *L. brevis* and from 50 to 13 % for *L. plantarum*.

The Langmuir model is the most frequently used model to describe simple sorption isotherms and to compare the performance of different biosorbents. Although

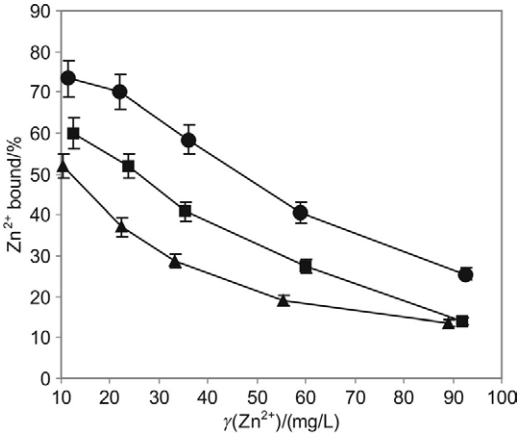

Fig. 1. Binding of zinc from aqueous solutions by LAB (0.8 g/L) after 24 h at pH=5 and temperature of 32 °C (● *L. mesenteroides*, ■ *L. brevis*, ▲ *L. plantarum*)

this model does not describe complete sorption process (it could be applied only for monolayer binding of metal ions to bacterial cell wall model), it was used here to elucidate adsorption differences among bacterial species. During the biosorption, a rapid equilibrium is established between the adsorbed metal ions on the bacterial cell (q_{eq}) and the unadsorbed metal ions in the solution (c_{eq}). This equilibrium represented by the Langmuir adsorption isotherm was used in some studies to describe the biosorption of heavy metal ions by bacterial cells, yeast and algae. The Langmuir equation, which is valid for monolayer sorption, is given by Eq. 1:

$$q_{eq}=Q_o b c_{eq}/(1+bc_{eq}) \qquad /1/$$

where q_{eq} is the specific binding (mg/g of dry mass), Q_o is the maximum amount of the metal ion per mass unit of bacteria to form a complete monolayer on the surface bound at high c_{eq} (mg/L), b is a constant related to the affinity of the binding sites, and c_{eq} is the free concentration of metal ions at equilibrium. Q_o represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance. Q_o and b can be determined from linear plot of c_{eq}/q_{eq} *vs.* c_{eq}. The binding isotherms were generated for all tested species (Fig. 2) and estimated model parameters are shown in Table 1.

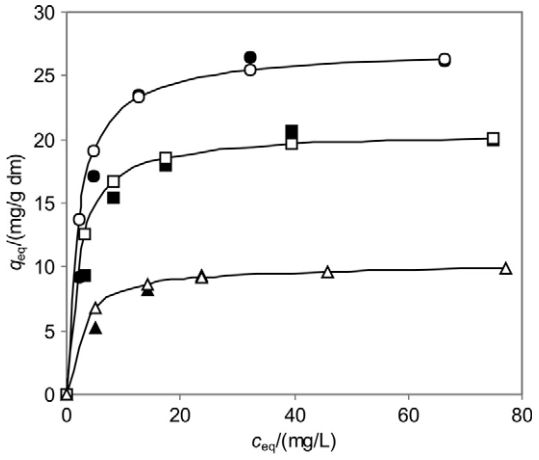

Fig. 2. Langmuir isotherms for zinc binding from aqueous solutions by 0.8 g/L of LAB after 24 h at pH=5 and temperature of 32 °C (● *L. mesenteroides*, ■ *L. brevis*, ▲ *L. plantarum*; black symbols represent experimental data, and white symbols represent a Langmuir model)

Table 1. Langmuir model parameters for the binding isotherms of zinc by LAB in aqueous solutions

Bacterium	Q_o	b	R^2
L. mesenteroides	27.10±1.1	0.40±0.05	0.9968
L. brevis	20.70±1.0	0.50±0.04	0.9971
L. plantarum	10.18±1.8	0.49±0.01	0.9972

Binding of zinc from the aqueous solutions by LAB (0.8 g/L) after 24 h at pH=5 and temperature of 32 °C

Q_o=maximum binding capacity, b=affinity between the sorbent and the sorbate

Q_{max} value of *L. mesenteroides* was 27.10 mg/g, somewhat higher than that achieved with *L. rhamnosus*, but lower than with *Propionibacterium freudenreichii*, for the binding of cadmium and lead (*18*). Treatment of bacterial biomass with heating or exposure to acid or base can enhance metal ion binding by microorganisms, due to the increase of metal binding sites on the cell wall. However, in this work bacterial biomass was not treated before any experiment was conducted, because of possible human diet supplementation with probiotically active zinc-enriched LAB biomass.

Influence of external conditions on and mechanism of binding zinc ions by LAB

Environmental physicochemical parameters can significantly influence metal binding by a microorganism. Therefore, the biosorption of zinc ions by different species of LAB was investigated as a function of pH, biomass concentration, and temperature. Earlier studies on metal ion biosorption had shown that pH was the single most important parameter affecting the biosorption process (*19*). The results presented in Fig. 3 support this assumption, because the initial pH solution significantly affected the zinc binding by *L. mesenteroides* ($p \leq 0.05$). At lower pH values, affinity of the cell wall for metal ions decreased, presumably due to the competition with H^+ ions for the binding sites. An enhanced adsorption at higher pH could be due to an increase in the negative charge of surface functional groups. At pH higher than 6, zinc was found to precipitate in the control samples. Because of that, experiments at pH≥6 were excluded and all other experiments were performed at pH=5.

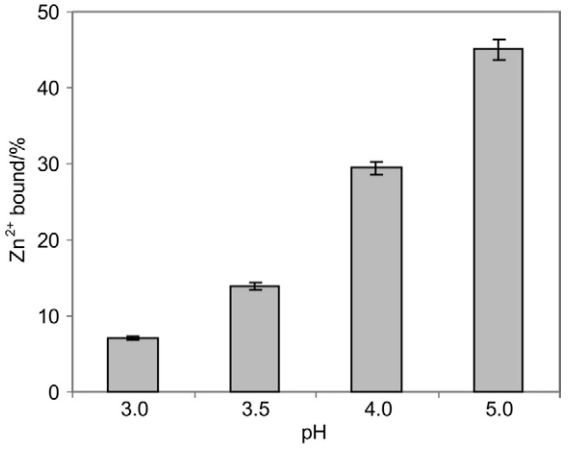

Fig. 3. Influence of the pH of aqueous solution on zinc ion binding by 0.5 g/L of *L. mesenteroides* after 24 h at 32 °C and initial zinc ion concentration of 20 mg/L

The results presented in Fig. 4 show that bacterial concentration strongly affected the amount of bound zinc ions. Increased concentration of *L. mesenteroides* increased the amount of zinc bound to bacterial biomass, although it had been reported that the aggregates formed during biosorption at higher biomass concentration reduced the efficiency of adsorption (*26*). It was found that zinc ion binding followed linear pattern and increased from 25 % at 0.55 mg/L of dried bacterial bio-

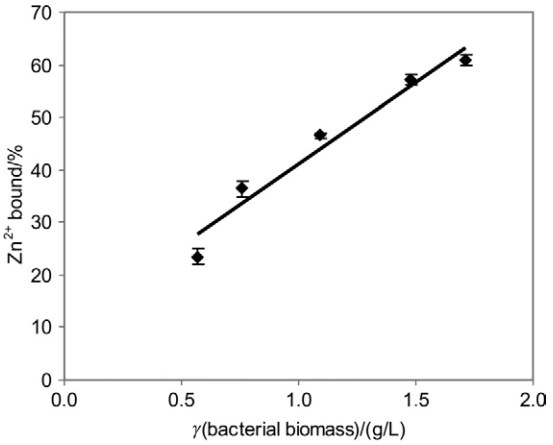

Fig. 4. Influence of biomass concentration on zinc ion binding by *L. mesenteroides* after 24 h at 32 °C and pH=5 and initial zinc ion concentration of 50 mg/L

mass to 60 % at 1.7 mg/L dm. The increase in zinc binding with increasing the biomass may be explained by a higher number of binding sites. Although biosorption is defined as initial rapid accumulation step in metal ion binding that is metabolism- and temperature-independent, in this work the temperature significantly increased the percentage of zinc bound to LAB. The binding of zinc ions from aqueous solution at 20 mg/L by *L. mesenteroides* (0.7 g/L dm) after 24 h at different incubation temperatures is shown in Fig. 5. The results show that the incubation temperatures significantly affected the zinc binding by *L. mesenteroides* ($p \leq 0.001$). These results are in agreement with the findings of Halttunen *et al.* (*17*). They reported that temperature affects the removal of Cd by *L. rhamnosus* GG, and that binding of Cd increased with the temperature increase. Temperature changes may affect the stability of the metal-microorganism complex, which depends on the biosorption sites, wall configuration of the microorganism cell, and the ionization of chemical moieties on the cell wall.

For further determination of zinc ion binding mechanism, the influence of contact time on zinc binding by LAB was investigated. Metal ion accumulation often

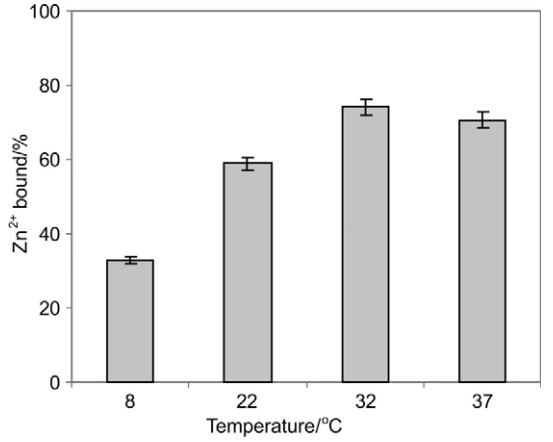

Fig. 5. Influence of temperature on zinc ion binding by 0.7 g/L of *L. mesenteroides* after 24 h at pH=5 and initial zinc concentration of 20 mg/L

comprises two stages: initial fast and passive process involving physical adsorption or ion exchange at cell surfaces, and slower transport of metal ions into bacterial cells. Zinc ion binding process (Fig. 6) also followed this pattern. Within the first phase, the first 3 hours, the major part of zinc ions was bound. However, upon prolonged incubation, more zinc was bound. Because of that, we can conclude that zinc ion binding is not coupled with cell wall only, and that intracellular structures also take place in this process. These results were confined by electron microscopy (Fig. 7). Electron microscopic examinations of *L. mesenteroides* were performed before and after metal ion binding in order to investigate the mechanism and location of zinc ions in *L. mesenteroides* cell. It was obvious that the major part of zinc ions was coupled with cell wall, but the zinc binding process involved zinc internalization into the cell too.

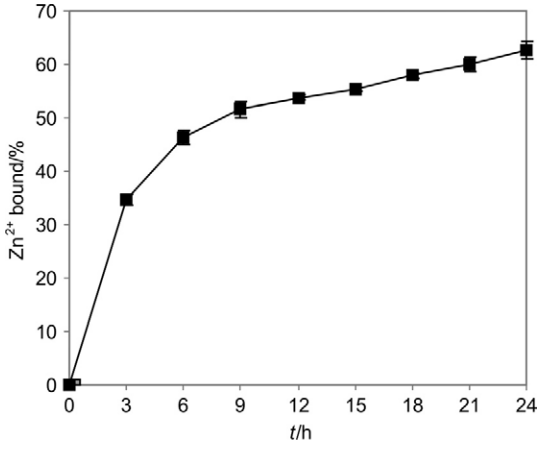

Fig. 6. Binding of zinc from aqueous solution (20 mg/L) at pH=5 by *L. mesenteroides* (0.8 g/L dm) after 24 h at different incubation times

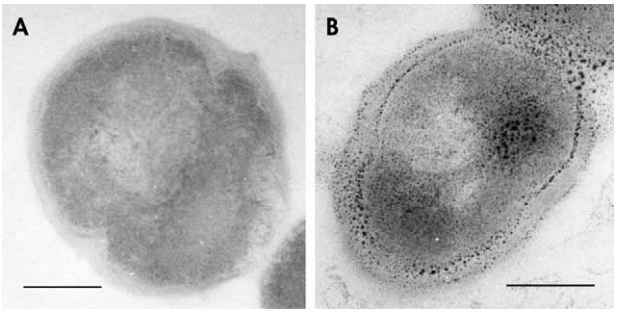

Fig. 7. Electron micrographs of (A) control *L. mesenteroides* and (B) *L. mesenteroides* exposed to zinc ions. Bar=0.2 μm

The roles of various functional groups in zinc binding can be defined by FT-IR spectroscopy (*27*). FT-IR spectra of microorganisms are usually divided into five regions (*28*). These regions contain information on different cell components: 3000–2800 cm^{-1}: fatty acids in the bacterial cell membrane, 1800–1500 cm^{-1}: amide bands from proteins and peptides; 1500–1200 cm^{-1}: region of proteins and fatty acids; 1200–900 cm^{-1}: polysaccharides

within the cell wall; and 900–500 cm^{-1}: region containing bands which cannot be assigned to special functional groups. The FT-IR spectra of blank and LAB loaded with zinc are presented in Fig. 8. The main goal of this work was to obtain zinc ions in the form of metalloprotein complex and because of that a specific region of proteins, peptides and polysaccharides within the cell wall (2000–500 cm^{-1}) was chosen.

The spectrum of the blank biomass displays several typical functional groups; distinct and sharp adsorptions at 1656 cm^{-1} (N-H bending) and 1544 cm^{-1} (C-O stretching) indicate amide I and amide II band, respectively, of the amide bond in *N*-acetylglucosamine polymer or the protein peptide bond; 1455 cm^{-1} indicates CH_3 bending in proteins. Absorptions at 1388 and 1234 cm^{-1} indicate O-H bending and C-O stretching bands of the carboxylate ion group (COO^-). Also, distinct absorption peak that can be seen at 1055 cm^{-1} indicates hydroxyl groups from saccharides. In comparison between the blank and *L. mesenteroides* loaded with zinc (Fig. 8), it was observed that there was some shift in FT-IR spectra due to metal binding process with bacterial biomass. After the contact with zinc, the biomass exhibited a spectrum with a clear shift in the region of proteins. The change in the band intensity in protein region, especially the band of carboxylate ion group, indicates the role of proteins in zinc binding.

It has been established that active uptake is most likely inhibited by low temperatures (*13*). Donocik *et al*. (*29*) reported rapid initial process of zinc binding by *Cytophaga johnsonae* and slow active accumulation in the media with the presence or absence of glucose. Based on our results and the literature data, it can be concluded that passive adsorption on cell wall and active uptake of zinc ions into the cytoplasm due to endogenous metabolism are involved in zinc binding by *Leuconostoc mesenteroides*, *Lactobacillus brevis* and *Lactobacillus plantarum*. Scott *et al*. (*30*) reported two FNR homologues, FlpA and FlpB, which control the expression of high and low affinity ATP-dependent Zn(II) uptake systems in *Lactococcus lactis*.

The cell wall constituents have a primary role in metal ion binding. The Gram-positive cell wall of LAB consists mainly of peptidoglycans, (lipo)teichoic acids, proteins and polysaccharides (*31*). The peptidoglycan consists of linear polysaccharide chains which consist of alternating *n*-acetylglucosamine and *n*-acetyl-muramic acid units extensively crosslinked by two short peptides. The polysaccharides associated with the bacterial cell wall and the extracellular polysaccharides of lactic acid bacteria are either neutral or acidic. Because of their abundance and their presence on the outer surface of the cell wall, extracellular and cell-wall associated polysaccharides are expected to determine to a large extent the surface properties of microorganisms. The most abundant surface proteins in many *Lactobacillus* species are the S-layer proteins (*32*). These compounds possess numerous functional groups including carboxylate, hydroxide, amine, phosphate, and hydrosulphide with various charge distributions, so they can selectively bind metal ions. The complex formation, ion exchange, adsorption, chelation and microprecipitation are the proposed mechanisms involved in metal ion binding. The dependence of zinc binding by LAB on the pH indicates that ion exchange is a mechanism responsible for the observed zinc binding. Vieira and Volesky (*33*) suggested the involvement of carboxyl grups of peptidoglycan, carboxyl and phosphate groups of teichoic acids in metal complexation after various chemical modifications of *B. subtilis* cell wall. From the results presented in Fig. 8, it can be concluded that carboxylate group of proteins has the most important role in zinc binding by *L. mesenteroides*. The results are in line with those reported by Lin *et al*. (*34*). They concluded that hydroxyl and carboxylate functional groups play a leading role in Ag^+ binding by *Lactobacillus* sp. A09. Also, carboxyl and phosphoryl groups have a significant role in binding of cadmium and lead by *L. fermentum* ME3 (*20*).

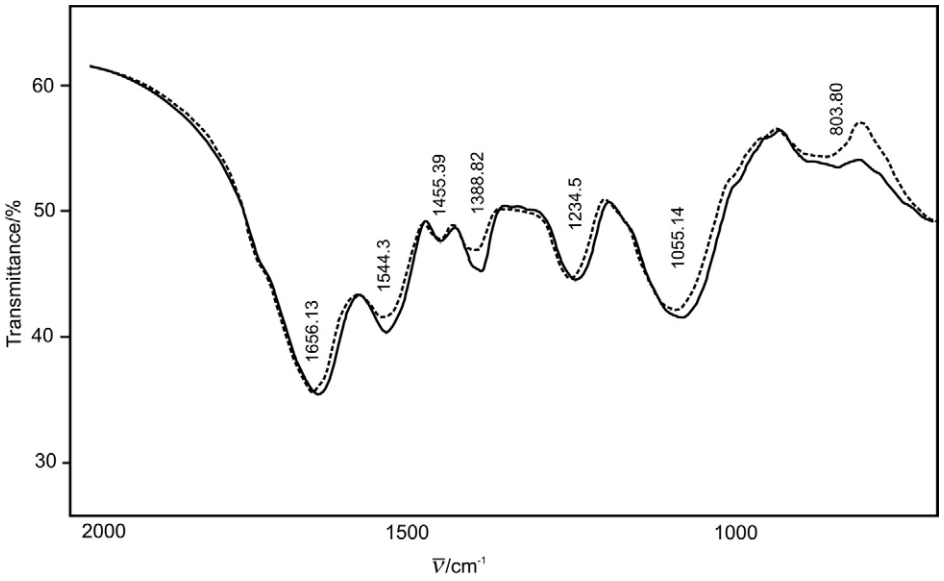

Fig. 8. *L. mesenteroides* FT-IR spectra of blank biomass (solid line) and biomass loaded with 20 mg/L of zinc after 24 h at pH=5 and temperature of 32 °C (dashed line)

Stability of metalloprotein complex in simulated gastric juice

Metalloproteins are easy and safe to be absorbed by humans and their absorption rates are higher than in inorganic state (*14*). Difficulties involved in studying bacterial properties in gastrointestinal tract, especially in humans, have led to the development of different model systems that simulate gastrointestinal tract. We used one of the model systems to study the behaviour of zinc-enriched LAB in gastric juice, and the results are presented in Fig. 9. As it can be seen, the zinc ion-cell bond in simulated gastric juice was unstable, independent of the bacterial species. About 95 % of zinc ions were discharged from the LAB, probably due to low pH value.

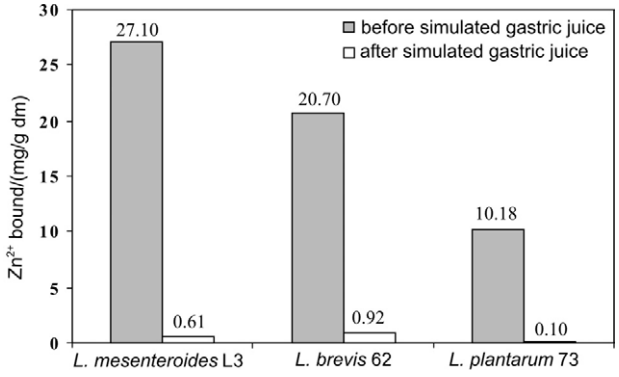

Fig. 9. Discharge of zinc ions in gastrointestinal model system. Biomass was centrifuged and exposed to simulated gastric juice for 4 h at pH=2.5 and 32 °C

Desorption of bound metals from the bacterial biomass is important for the practical applicability. That is why it is necessary to research a protection of zinc-enriched bacterial biomass that passes through the stomach to reach duodenum and jejunum, where the zinc absorption takes place. The microencapsulation seems to be a good solution, and this will be the next step of our investigations.

Zinc ions did not have toxic effect on the examined LAB during biosorption process (data not shown). Cell viability in simulated gastric juice was reduced for about 25 %, but the released zinc ions did not have any toxic effect (data not shown), contrary to copper ions, which significantly reduced cell viability (*35*). Contrary to that, Mudroňová *et al*. (*36*) achieved significantly enhanced probiotic effect of *L. plantarum* with the addition of zinc ions. With the addition of zinc propionate to *L. plantarum*, the number of *E. coli* cells in the laboratory mouse microflora was strongly reduced.

Conclusions

Zinc ions can be successfully bound by LAB. Zinc tended to be bound from the test solutions in a LAB-dependent manner and *L. mesenteroides* showed the highest ability for zinc ion binding. Differences among the species in binding performance were evaluated with Langmuir model of biosorption. Binding of zinc was influenced by ionic strength, pH, biomass concentration, and temperature. FT-IR spectroscopy analysis and electron microscopy have shown that passive adsorption on the carboxylate group of proteins, and active uptake of the zinc ions into the cytoplasm are involved in zinc binding.

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