Rapid removal of lead and cadmium from water by specific lactic acid bacteria

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Abstract

Cadmium and lead are highly toxic metals. People are exposed to them primarily through food and water. Available conventional methods (precipitation, flocculation, ion exchange, and membrane filtration) for removal of these metals from water at low concentrations are claimed to be expensive and inefficient. Different microbes have been proposed to be an efficient and economical alternative in heavy metal removal from water. In this work, specific lactic acid bacteria (LAB) were assessed for their ability to remove cadmium and lead from water. Significant removal was observed, and it was found to be metal and bacterial strain specific. Removal was a fast, metabolism-independent surface process. It was also strongly influenced by pH, indicating that ion exchange mechanisms could be involved. The most effective metal removers were Bifidobacterium longum 46, Lactobacillus fermentum ME3 and Bifidobacterium lactis Bb12. The highest maximum cadmium and lead removal capacities of 54.7 mg metal/g and 175.7 mg/g dry biomass, respectively, were obtained with B. longum 46.

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1. Introduction

Cadmium and lead are both highly toxic metals. Oral exposure to Cd may cause renal damage (Satarug and Moore, 2004), osteoporosis (Staessen et al., 1999; Järup and Alfvén, 2004), and possibly prostate (Waalke, 1991) and renal (Waalke et al., 1999) cancer. Chronic exposure to even low levels of cadmium could also lead to adverse renal (Järup et al., 2000) and negative bone effects (Staessen et al., 1999; Järup and Alfvén, 2004). Lead, on the other hand, disturbs hemo-globin synthesis, renal function and causes neurological and behavioural disturbances in children (WHO, 1995). Even low blood lead concentrations have been associated with intellectual impairment in children (Canfield et al., 2003).

Food is the most important source of cadmium exposure among the non-smoking population. In addition, especially children are exposed to lead via ingestion of dust, soil and lead-containing paints. Lead (Wyatt et al., 1998; Fertmann et al., 2004), but rarely cadmium, is found in drinking water at concentrations over guidelines set by WHO (2004) (10 μg/l for Pb and 3 μg/l for Cd). Lead contamination of drinking water is often a result from corrosion of lead-containing plumbing. Cadmium and lead are both also found in considerable concentrations as a result of point contamination, e.g. from industry.

Removal of heavy metals from (drinking) water can be achieved with precipitation, flocculation, ion exchange, and membrane filtration. These methods are sometimes expensive, not effective at low metal concentrations, and produce sludge to be disposed. Thus, safe novel treatments should be searched for future decontamination targets.

Probiotic bacteria have the capacity to bind many toxic compounds like aflatoxins (Peltonen et al., 2001; Haskard et al., 2001), food-borne mutagens (Turbic et al., 2002) and microcystin-LR (Meriluoto et al., 2005) from aqueous solution. There is also some evidence that probiotic bacteria could bind aflatoxin B1 (El-Nezami et al., 2000, 2006) and the food-borne mutagen Trp-P-2 (Ornhage et al., 2002) within the gastro-intestinal tract, thereby reducing their uptake. We have demonstrated in our pilot study that probiotic LAB bind cadmium from water (Halttunen et al., 2003). Therefore,
LAB could prove to be an effective tool in reducing heavy metal exposure.

In this work, we have assessed the ability of three Lactobacillus strains, three Bifidobacterium strains and two commercial starter culture preparations to bind lead and cadmium from water. In addition, the impact of physical and chemical conditions on binding was characterized.

2. Materials and methods

2.1. Bacterial strains

The following lactic acid bacteria (LAB) strains were used in this study: Lactobacillus rhamnosus GG (ATCC 53103) (Valio Ltd., Helsinki, Finland), Lactobacillus casei Shirota (Professor Y-K Lee, Yakult Singapore Pte. Ltd., Singapore), Lactobacillus fermentum ME3 (University of Tartu, Tartu, Estonia), B. longum 2C (Probiotical srl, Novara, Italy), B. longum 46 (Probiotical srl, Novara, Italy) and B. lactis Bb12 (Chr. Hansen Ltd., Hørsholm, Denmark). In addition, two commercial starter cultures were used: FVDVS XT-303-eXact (Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris, Leuconostoc pseudomesenteroides and Lactococcus lactis subsp. lactis biowar. diacetylactis) (Chr. Hansen Ltd., Hørsholm, Denmark) and YO-MIX 401 (Streptococcus thermophilus and Lactobacillus bulgaricus) (Danisco Niebüll GmbH, Niebüll, Germany) which will be later referred to as S1 and S2, respectively.

2.2. Growth media and culture conditions

L. casei Shirota, L. fermentum ME3 and S1 were cultured in MRS-broth (02–135, Scharlau Chemie S.A., Barcelona, Spain) for 48 h at +37 °C. Biomass was then centrifuged (8000 ×g, 15 min) and washed twice with ultra-pure water (Milli-Q plus, Millipore S.A., Molsheim, France). Washed biomass was lyophilised and stored at −20 °C. L. rhamnosus GG, B. longum 2C, B. longum 46, B. lactis Bb12 and S2 were cultured in commercial lyophilised and boiled (1 h) form. Here, conditions were selected, based on studies described above. All experiments were repeated at least twice.

2.3. Binding assay

The effect of contact time was investigated with all strains. Suspensions of lyophilised bacteria (2 g/l) were spiked with Pb²⁺ (Pb(NO₃)₂, 15314, Fluka Chemie GmbH, Buchs, Switzerland) or Cd²⁺ (Cd(NO₃)₂, 20895, Fluka Chemie GmbH, Buchs, Switzerland) and diluted to a final bacterial concentration of 1 g/l and a final metal concentrations of 50 and 10 mg/l for Pb²⁺ and Cd²⁺, respectively. The pH of the biomass–metal solution was adjusted to 5 in Pb²⁺ and 6 in Cd²⁺ experiments with dilute NaOH and HNO₃ and kept constant for the first 5 min. Three 1.5 ml samples were taken from the suspension and these were incubated for 5–240 min at +37 °C. After incubation, the pH of the suspension was measured and bacteria were separated by centrifugation (7000 ×g, 5 min). One milliliter of supernatant was preserved with 200 µl of ultra-pure nitric acid (17078, Fluka Chemie GmbH, Buchs, Switzerland) and stored at room temperature.

The effect of pH (2–6), bacterial concentration (0.5–1.5 g/l) and temperature (+4, 22 and 37 °C) was tested according to the method described above. The effect of pH was studied with L. rhamnosus GG, L. fermentum ME3, B. longum 46 and B. lactis Bb12. The effect of bacterial concentration and temperature was studied only with L. rhamnosus GG.

The effect of metal concentration (0.1–100 mg/l) on cadmium and lead binding was determined with all strains in lyophilised and boiled (1 h) form. Here, conditions were selected, based on studies described above. All experiments were repeated at least twice.

2.4. Measurements of cadmium and lead

Lead and cadmium concentrations were determined with atomic absorption spectrometry (Solaar M6 Dual Zeeman AAS Spectrometer, Thermo Electron Spectroscopy Ltd., Cambridge, England) either by flame or graphite furnace method depending on the metal concentration. In each analysis, samples spiked with lead and cadmium were used as quality control samples.

2.5. Data analysis

Experimental results from metal removal studies were fitted to theoretical model which enables calculation of specific descriptive parameters. In this work, metal removal ability of different LAB strains was evaluated using a Langmuir isotherm described in Eq. (1) (Davis et al., 2003).

\[ q = \frac{q_{\text{max}} bC_f}{1 + bC_f} \]

(1)

Plotting \( C_f \) (free concentration of metal in equilibrium state) and \( q \) (bound concentration of metal in equilibrium state), which are obtained from experimental data, enables the calculation of \( q_{\text{max}} \) (maximum binding capacity at given conditions) and \( b \) (coefficient related to the initial slope of the curve and to the affinity of binding) from Eq. (1) (see Fig. 1).

![Fig. 1. Effect of metal concentration on cadmium binding with B. longum 46. Mean of two replicates and standard deviation is shown. Two lines illustrate the theoretical parameters, maximum binding capacity of the biomass (\( q_{\text{max}} \)) and affinity of binding (\( b \), calculated from the Langmuir isotherm.)](image-url)
3. Results

Binding of both Cd\(^{2+}\) and Pb\(^{2+}\) occurred rapidly with all studied strains. After 5 min of incubation, binding of cadmium (10 mg/l) at pH 6 and lead (50 mg/l) at pH 5 ranged between 61.8\(\pm\)3.3\% – 87.8\(\pm\)2.9\% and 30.2\(\pm\)7.9\% – 92.6\(\pm\)1.9\%, respectively. With most of the strains, further incubation had no effect on the binding. However, in the case of *L. casei* Shirota, cadmium removal increased from 61.8\(\pm\)3.3\% after 5 min to 74.5\(\pm\)3.3\% after 4 h while the amount of cadmium removed by *L. fermentum* ME3 decreased from 81.2\(\pm\)5.3\% after 5 min to 62.2\(\pm\)3.4\% after 4 h. Based on these results, a contact time of 1 h was chosen for further studies to ensure equilibrium conditions, which are a prerequisite for use of the Langmuir isotherm. In order to simplify the comparison of different strains, the same contact time was also chosen for lyophilised *L. casei* Shirota and *L. fermentum* ME3.

During the incubation of samples, a small drop in pH was observed. This was found in all experiments except in the pH experiment at low pH values.

The cadmium and lead removal assessment indicated a strongly pH-dependent process with the highest binding at a pH close to neutral (Figs. 2 and 3). The impact of pH on cadmium removal with all strains studied was quite similar. Removal was negligible at pH \(\leq\) 3 with *L. rhamnosus* GG, *L. fermentum* ME3 and *B. longum* 46, and about 20\% with *B. lactis* Bb12 at pH 2. Increasing pH caused an almost linear increase in removal, and the highest binding of cadmium (60\%–73\%) was achieved at pH 6. Removal of lead with *L. rhamnosus* GG, *L. fermentum* ME3 and *B. lactis* Bb12 followed a pattern similar to that of cadmium, although the increase in binding with ME3 started at lower pH than with the other strains. With *B. longum* 46 the removal of lead was high (55\%) already at pH 2. The binding increased linearly as a function of pH, and the highest removal (95\%) was achieved at pH 6. To avoid precipitation of metals as metal hydroxides, pH values higher than 6 were not tested. Based on these results, pH values of 6 (Cd\(^{2+}\)) and 5 (Pb\(^{2+}\)) were used in other experiments.

Increasing the bacterial concentration of *L. rhamnosus* GG enhanced the binding of cadmium and lead (Fig. 4). A concentration of 1 g/l was selected for other experiments. The effect of temperature on both lead and cadmium binding by *L. rhamnosus* GG was small (data not shown), and thus, other experiments were performed at 37 °C.

All bacterial strains studied were effective in removing low concentrations of cadmium and lead in water. Up to 99\% of cadmium and 97\% of lead were removed from solutions with initial metal concentrations of 100 and 1000 \(\mu\)g/l. Specific binding (mg metal bound/g dry biomass) of cadmium and lead increased when the initial metal concentration was increased, until a saturation point was reached (Fig. 1). The maximum cadmium binding \((q_{\text{max}})\) calculated from the Langmuir equation ranged from 12.1 to 54.7 mg/g (Table 1). Lead binding was higher, maximum values ranging from 32.3 to 175.7 mg/g. The affinity of cadmium binding \((b)\) varied from 0.05 to 0.51 l/mg. For lead, the range was from 0.03 to 0.59 l/mg. Only boiled *B. lactis* Bb12 in lead binding did not fit to this model. Otherwise, the Langmuir model described cadmium and lead binding well. Lead binding by boiled *B. lactis* Bb12 was low at low metal concentrations but increased steeply at higher concentrations. The most efficient strains in both cadmium and lead binding were *B. longum* 46, *B. lactis* Bb12 and *L. fermentum* ME3. In general, boiling had only a minor effect on the maximum binding capacities \((q_{\text{max}})\) of cadmium and lead. A clear difference was only seen with

\[\text{Fig. 1. Effect of pH on cadmium binding by different LAB strains. Mean of at least two replicates and standard deviation are shown. Initial } c(\text{Cd}^{2+}) = 10 \text{ mg/l.}\]

\[\text{Fig. 2. Effect of pH on cadmium binding by different LAB strains. Mean of at least two replicates and standard deviation are shown. Initial } c(\text{Cd}^{2+}) = 10 \text{ mg/l.}\]

\[\text{Fig. 3. Effect of pH on lead binding by different LAB strains. Mean of at least two replicates and standard deviation are shown. Initial } c(\text{Pb}^{2+}) = 50 \text{ mg/l.}\]

\[\text{Fig. 4. Effect of bacterial concentration on metal removal by *L. rhamnosus* GG. Mean of at least two replicates and standard deviation are shown. Initial } c(\text{Cd}^{2+}) = 10 \text{ mg/l and } c(\text{Pb}^{2+}) = 50 \text{ mg/l.}\]
The results demonstrate that cadmium and lead binding by safe and food grade lactic acid bacteria and bifidobacteria took place rapidly from aqueous solution. Similar results have been reported for other bacteria, like Bacillus subtilis (Fein et al., 1997) and Pseudomonas putida (Pardo et al., 2003). The quick uptake suggests that binding occurred passively to the surface of bacteria rather than by accumulation inside the cell.

Cadmium and lead removal by the studied LAB was enhanced at higher pH. Similar effects of pH on cadmium and lead removal have been observed for other bacteria, like Bacillus subtilis (Fein et al., 1997), P. putida (Pardo et al., 2003) and a Citrobacter strain (Puranik and Paknikar, 1999). Also magnesium binding with isolated teichoic acids of L. buchneri N.C.I.B. 8007 was affected similarly by pH (Lambert et al., 1975). The effect of pH probably results from competition for negatively charged binding sites between heavy metal cations and protons (H⁺) (Huang et al., 1991). Also cationic metal ions (Ca²⁺ and Mg²⁺) are reported to compete with Cd²⁺ in a similar manner (Brady and Tobin, 1995). The higher lead removal at low pH, observed especially with B. longum 46, could be a result of a higher number of available phosphate groups on the bacterial surface. These groups are mainly in unprotonated form already at pH 2 (pKₐ = 1.5) (Huang et al., 1991). The efficient lead removal with this strain could also result from a different binding mechanism. Yun and Volesky (2003) have postulated that the complexation of cadmium to phosphonate groups could take place also when these groups are in protonated form at low pH.

Mechanisms such as complex formation, ion exchange, adsorption, chelation and microprecipitation, have been proposed to be involved in metal biosorption. The dependence of pH in our work indicates that ion exchange is probably at least partly responsible for the observed metal binding. The observed drop in pH during the incubation, which is probably a result of proton replacement by heavy metal ions, supports this conclusion. Involvement of anionic surface groups in metal binding has been reported for the Gram-positive bacterium, B. subtilis. Extraction of the teichoic acid moieties (phosphodiesters) and reduction of the number of free carboxylic groups, reduced the cation uptake by isolated B. subtilis cell walls (Beveridge and Murray, 1980; Doyle et al., 1980). The surface of LAB, like other Gram-positive bacteria, is composed of a thick layer of peptidoglycan, teichoic acids, protein and polysaccharides (Delcour et al., 1999). These structures contain different kinds of charged groups like carboxyl, hydroxyl and phosphate groups. Lactic acid bacteria have therefore a great number of different possible ligands capable of binding cationic ions like cadmium and lead.

The increase in metal removal with increasing biomass may be explained by a higher number of binding sites. Incubation

### Table 1
Parameters (q_max and b) obtained from the Langmuir isotherm for Cd (pH 6) and Pb (pH 5) binding of LAB at +37 °C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cd</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>q_max</td>
<td>b</td>
</tr>
<tr>
<td>B. lactis Bb12</td>
<td>32.1±1.3</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td></td>
<td>34.1±0.9</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>B. longum 2C</td>
<td>14.6±0.3</td>
<td>0.15±0.06</td>
</tr>
<tr>
<td></td>
<td>13.7±1.4</td>
<td>0.33±0.04</td>
</tr>
<tr>
<td>B. longum 46</td>
<td>32.0±2.6</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td></td>
<td>54.7±3.5</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>L. casei Shirota</td>
<td>19.0±1.5</td>
<td>0.51±0.11</td>
</tr>
<tr>
<td></td>
<td>12.1±2.2</td>
<td>0.51±0.17</td>
</tr>
<tr>
<td>L. fermentum ME3</td>
<td>26.7±0.2</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td></td>
<td>28.4±3.7</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>L. rhamnosus GG</td>
<td>12.5±4.2</td>
<td>0.32±0.23</td>
</tr>
<tr>
<td></td>
<td>13.2±2.2</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Starter 1</td>
<td>21.9±2.1</td>
<td>0.22±0.03</td>
</tr>
<tr>
<td></td>
<td>23.3±0.6</td>
<td>0.51±0.13</td>
</tr>
<tr>
<td>Starter 2</td>
<td>25.9±0.5</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td></td>
<td>28.2±0.5</td>
<td>0.25±0.04</td>
</tr>
</tbody>
</table>

Mean of at least two replicates and standard deviation are shown.

- a Maximum binding capacity (mg metal bound/g dry biomass).
- b Coefficient related to affinity of binding (l/mg).
- c Regression coefficient.
- d Boiled biomass.
- e Freeze-dried biomass.
- f Data did not fit to the model.
- g FV-DVS XT-303-eXact (L. lactis subsp. cremoris, L. lactis subsp. lactis, L. mesenteroides subsp. cremoris, L. pseudomesenteroides and L. lactis subsp. lactis biovar. diacetylactis).

B. longum 46 in cadmium and lead binding, and with L. rhamnosus GG in lead binding.

### 4. Discussion

The results demonstrate that cadmium and lead binding by safe and food grade lactic acid bacteria and bifidobacteria took place rapidly from aqueous solution. Similar results have been reported for other bacteria, like Bacillus subtilis (Fein et al., 1997) and Pseudomonas putida (Pardo et al., 2003). The quick uptake suggests that binding occurred passively to the surface of bacteria rather than by accumulation inside the cell.

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The increase in metal removal with increasing biomass may be explained by a higher number of binding sites. Incubation
temperature (+4–37 °C) had no effect on metal removal, indicating that the binding process is energy-independent (data not shown). Similar observations were made with a Citrobacter strain in lead, cadmium and zinc binding (Puranik and Paknikar, 1999), and with yeast biomass in lead binding (Cho and Kim, 2003).

Metal binding in this work followed well the Langmuir isotherm, except in the case of boiled B. lactis Bb12. Binding following the Langmuir model also has been observed for many other microorganisms (Volesky and Holan, 1995). Compared to other microorganisms studied, cadmium and lead binding by LAB is moderate. For example, maximal removal capacities as high as 131.0 mg/g (Cd²⁺) (Holan et al., 1993) and 252.0 mg/g (Pb²⁺) (Holan and Volesky, 1994) have been reported for brown seaweed Sargassum natans. We observed that the removal of lead was higher than cadmium. This may result from bigger ionic radius of lead than cadmium (Davis et al., 2003). The preference for certain metals over others has also been reported for other microorganisms (Brady and Tobin, 1995; Davis et al., 2003).

In conclusion, LAB and bifidobacteria have been demonstrated to bind toxic metals efficiently and rapidly in water. Since LAB are also known to efficiently bind microcystin-LR from water and dietary mutagens and aflatoxins in food, water and inside the gastro-intestinal tract, they appear to be effective decontamination tools. There is a need for further experiments to assess their value in water purification, and if similar binding could actually take place in vivo. Based on this work, B. longum 46 and L. fermentum ME3 were selected for further testing.

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