Diet is the major source of cadmium for the non-smoking population. Currently, no method is available to remove cadmium from food products. Because the content of cadmium in the food is likely to continue to rise in the future, a new method for decontaminating foods is urgently needed. We assessed the ability of safe food grade lactic acid bacteria (LAB) to remove cadmium from an aqueous solution. At a concentration of 10 µg/l up to 70% could be removed within 5 min, and up to 90% after 1 hr. At a concentration of 1000 µg/l between 5 and 30% was removed after 5 min and between 20 and 55% after 1 hr. Heat-treatment of the bacteria significantly enhanced the removal of Cd at 10 and 100 µg/l, but not at 1000 µg/l. Increased temperature (37°C), prolonged incubation (24 hr) and higher bacterial concentrations (5 x 10⁹ bacteria/ml) were found to increase the removal of Cd. LAB were shown to remove cadmium in a strain, temperature and concentration dependent manner. The results indicate that food grade LAB may provide a much needed means for decontamination of liquid foods. The practical feasibility of this approach deserves to be further investigated considering the importance of Cd removal from food.

Key words: lactic acid bacteria; cadmium; heavy metal

INTRODUCTION

Cadmium (Cd) finds its way into the environment from both natural and human sources. Industrial emissions and the use of phosphate fertilizers are thought to be responsible for increasing levels of cadmium in the soil. Increased Cd levels in the soil in turn lead to an increased uptake of cadmium by the crop. Cd is therefore present in most foods, and diet is the major source of cadmium in the general non-smoking population (16). The Cd content of wheat has more than doubled between 1918 and 1980 (2). Because the Cd content of soil is expected to continue to increase by 0.2 to 0.6% annually (1, 14), the Cd content of foods can also be expected to rise. Despite the significant health risks associated with Cd exposure and the expected increase in exposure from dietary sources, no method is currently available to treat Cd contaminated foods or fertilizers. Specific bacteria have been observed to be able to absorb certain metal ions (11, 13). This principle has only been investigated from an environmental point of view to decontaminate or recover heavy metals from industrial aqueous process streams (13, 17). Therefore, mainly soil bacteria have been investigated (9). In the current study, we investigated the ability of lactic acid bacteria (LAB), which are widely used in food products, to remove Cd from aqueous test solutions. The ability of food grade microorganisms to remove Cd from liquid foods such as infant formulas and milk could be expected to have wide application (4).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The following LAB were selected for the study: Lactobacillus rhamnosus GG (ATCC 53103), Lactobacillus rhamnosus LC705 (Valio Ltd., Helsinki, Finland), Lactobacillus johnsonii Lj1 (isolated from a Nestlé LC1 product), Lactobacillus casei Shirota (Yakult Singapore Pty. Ltd., Singapore) and Bifidobacterium lactis Bb 12 (Chr. Hansen Ltd., Hørsholm, Denmark). These strains were selected because of their known extensive food use around the world (10), their documented health effects (3) and their safety record for human consumption (8). Bacteria were grown anaerobically at 37°C, for 48 hr in de Man, Rogosa and Sharp broth (MRS; Merck, Darmstadt) and thereafter washed twice with ultra pure water (Milli-Q plus, Millipore Corp., USA). Ultra pure water was chosen because NaCl present in phosphate buffered saline was found to cause high background signals with the Cd detection (data not shown). Before use in the Cd binding assay (see below) the bacterial concentrations were adjusted to 5 x 10⁹ bacteria/ml using flow cytometry (15). Bacterial suspensions were then divided into 2 ml aliquots, centrifuged (1700 x g, 5 min) and supernatants discarded. Part of the bacterial suspensions were boiled for 1 hr since this has earlier
been shown to improve aflatoxin B1 removal (6) and was therefore hypothesised to also affect the removal of Cd.

Cadmium removal assay. Bacterial pellets were resuspended into 2 ml ultra pure water containing the following concentrations of Cd (from Cd(NO3)2): 0, 10, 100 and 1000 µg/l. These concentrations are in the range of normal food residue levels (10-100 µg/l), while the highest level tested exceeds the current maximum residue limit 10 fold. The suspensions were mixed thoroughly and a 1 ml aliquot was removed immediately, of which the liquid phase was collected by centrifugation (t = 5 min), while the other half of the suspension was further incubated at 37°C for 1 hr. The suspensions were centrifuged (1700 x g, 5 min), and the supernatants stored at -20°C for Cd analysis. Negative controls containing only ultra pure water and negative controls containing bacteria in ultra pure water without any Cd additions and positive controls with Cd in ultra pure water were carried out with each experiment.

Because these results indicated that L. rhamnosus GG was one of the best Cd removing strains and because this strain has also been extensively investigated for its aflatoxin B1 removing capacity (5, 6), more detailed studies were performed on this strain only. The effect of the bacterial density on the removal of Cd at a concentration of 100 µg/l was investigated. L. rhamnosus GG was suspended at densities ranging from 5 x 10^7 to 5 x 10^9 bacteria/ml and incubated for 1 hr at 37°C. The rest of the experiment was performed as out-lined above.

The effect of the incubation temperature on the removal of Cd at a concentration of 100 µg/l was investigated. L. rhamnosus GG was suspended at concentrations of 5 x 10^8 bacteria/ml and incubated 1 hr at 4, 22 or 37°C. The rest of the experiment was performed as out-lined above.

The effect of the incubation time on the removal of Cd at a concentration of 100 µg/l was investigated. L. rhamnosus GG was suspended at a concentration of 5 x 10^8 bacteria/ml and incubated 1 hr at 37°C for 5 min, 1, 2, 4, 8 and 24 hr. The rest of the experiment was performed as out-lined above.

Analysis of metals. Analysis of the Cd content in the supernatants was performed by Atomic Absorption Spectrophotometry (AAS; SpectrAA 300, GTA-96 graphite furnace, Varian Techtron Pty Ltd., Mulgrave, Australia) employing D2-background correction. Two commercial reference materials were used as quality control samples; GSV-3 poplar leaves and GBW08504 cabbage (Institute of Geophysical and Geochemical Exploration, Langfang, P.R. China).

Statistics. All Cd removal experiments were performed with three assays on separate occasions, each assay had three parallels to correct for intra assay errors. The Cd content of each sample was analyzed in duplicate. Analysis of variance (ANOVA) followed by the Bonferroni/Dunn procedure for multiple comparisons was performed to evaluate the statistical significance (p < 0.05) of differences in Cd removal.

RESULTS

Cd tended to be removed from the test solutions in a strain dependent manner, although the differences between the strains were not statistically significant. Removal ranged between 5 and 30% at the highest tested concentration (1000 µg/l) and between 15 and 71% at the lowest concentration (10 µg/l), when viable bacteria were separated from the liquid phase immediately after mixing (t = 5 min), Fig. 1A.

When viable bacteria were incubated for 1 hr in the Cd containing test solutions, the removal of Cd was improved for all tested strains, though this was not statistically significant (Fig. 1B). Ranging from 29 to 54% of the Cd at the highest concentration and from 30 to 90% at the lowest concentration. Only Lactobacillus GG did not remove more Cd from all tested concentrations nor did B. lactis Bb12 at the 10 µg/l test solution after 1 hr incubation compared to 5 min. While L. casei Shirota did not exhibit a concentration dependent removal of Cd when incubated 1 hr, it was found to be constant at approximately 30% (p = 0.038; Fig. 1B).

When the bacteria were heat-treated, the removal of Cd was significantly (p ≤ 0.0002) enhanced for all stains at 10 and 100 µg/l after 5 min incubation as compared to viable bacteria (Figs. 1A and 2A). At the highest tested concentration, the removal of Cd was not significantly different between viable (Figs. 1A and 1B) or heat-treated bacteria (Figs. 2A and 2B). After 1 hr incubation, Cd removal was significantly enhanced for the boiled bacteria compared to the viable bacteria at 10 and 100 µg/l, with the exception of L. rhamnosus LC705 (Figs. 1B and 2B). No significant differences in Cd removal were observed between 5 min and 1 hr incubation with the heat-treated bacteria (p > 0.05).

Upon increasing concentrations of L. rhamnosus GG an increasing fraction of Cd (100 µg/l) was removed from the test solution, from 15% at 10^8 bacteria/ml to almost 90% at 2 x 10^8 bacteria/ml (Fig. 3).

The incubation temperature was found to significantly affect the removal of Cd (100 µg/ml) by L. rhamnosus GG from the test solution (p < 0.05). The removal increased from 47% at 4°C, and 56% at 24°C, to 64% at...
Fig. 1. Removal of cadmium from aqueous solutions by selected viable lactic acid bacteria (5 x 10⁸ bacteria/ml). The bacteria were incubated 5 min (A) or 1 hr (B). Three Cd concentrations were tested; 10, 100 and 1000 μg/l. GG, Lactobacillus rhamnosus GG; LC 705, L. rhamnosus LC705; LJ1, L. johnsonii LJ1; Shi, L. casei Shirota; Bb12, Bifidobacterium lactis Bb12. Results are the average of three independent observations.

Most of the Cd was removed by L. rhamnosus GG from the 100 μg/l test solution within the first few minutes (43%). However, upon prolonged incubation more Cd was removed, until 66% was removed after 24 hr incubation. The binding of the Cd was found to follow a logarithmic pattern: Cd% = 4.0896 ln(time) + 51.104 (R² = 0.95), where Cd% is the percentage of Cd present in the test solution after incubation and time is the incubation time (Fig. 4).

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Fig. 2. Removal of cadmium from aqueous solutions by selected heat-treated lactic acid bacteria (5 x 10⁸ bacteria/ml). The bacteria were incubated 5 min (A) or 1 hr (B). Three Cd concentrations were tested; 10, 100 and 1000 μg/l. GG, Lactobacillus rhamnosus GG; LC 705, L. rhamnosus LC705; LJ1, L. johnsonii LJ1; Shi, L. casei Shirota; Bb12, Bifidobacterium lactis Bb12. Results are the average of three independent observations.

DISCUSSION

Binding of Cd and other heavy metals from contaminated soil has been investigated for a number of soil bacteria (9, 11, 12). However, the use of safe food grade microorganisms or probiotics has not been investigated earlier. The ability of the test microbes to remove Cd, would have considerable potential use. Therefore, the ability to remove Cd from an aqueous solution was investigated for five commonly used food grade probiotic LAB.
Cd was observed to be removed in a strain dependent manner, where *L. rhamnosus* GG, *L. rhamnosus* LC705 and *B. lactis* Bb12 tended to have higher removal, though not significantly different from the other tested strains. It is of interest to note that both *L. rhamnosus* strains were also observed to give the highest aflatoxin B1 removal from test solutions (5, 6).

The absorption of Cd was also found to be concentration dependent. From the highest Cd concentration (1000 µg/l) a smaller fraction was removed than from the other concentrations tested. An exception to this was *L. casei* Shirotta which bound approximately 30% of all three tested concentrations, i.e. 27 pmol, 270 pmol and 2700 pmol/5 × 10⁸ bacteria. Thus, although a similar fraction of the Cd was removed, the total amount of Cd that was removed was bigger at higher concentrations, suggesting equilibrium kinetics of the removal. This indicates that *L. casei* Shirotta removes Cd by a different mechanism than the other tested strains.

Heat-treatment of the bacteria increased the amount of removed Cd compared to the live bacteria at 10 and 100 µg/l. Kurek and co-workers (9) observed a similar increase in Cd binding with ethanol and KCN-killed *Paracoccus* sp. and *Serratia marcescens*. While an increased removal of aflatoxin B1 by heat-treated LAB has also been observed (6). The observed increased removal of the Cd may relate to increased accessibility of Cd binding components on the bacterial cell after heat-treatment rather than to an increased number of Cd binding sites; prolonged incubation of heat-treated bacteria, 5 min vs. 1 hr, did not change the amount of Cd removed. A further indication that the number of Cd binding sites on the cells has not increased comes from the observation that after 1 hr incubation at the highest tested concentration (1000 µg/l) the amount of Cd removed by boiled bacteria was, in fact, reduced compared to live cells.

The logarithmic pattern of Cd binding over time, is typical for the absorption of metal ions by bacteria (8) and could relate to the accessibility of Cd binding sites on the bacteria. Also the increased binding after 1 hr incubation at higher temperatures could relate to this since diffusion will be increased at higher temperatures.

The mechanism of the Cd removal remains to be further investigated, though it is likely that the Cd is associated with structures of the bacterial cell envelope. This has been reported for *Bacillus subtilis*, *B. cereus* and *Pseudomonas aeruginosa* (11).

For practical applications, the use of immobilized heat-treated bacteria for removal of Cd from liquid foods as infant formulas and milk could be considered. With sufficiently high bacterial densities, 90–95% of the Cd can be removed from solutions with contact times as short as 5 min. An alternative application would be the consumption of the bacteria which could absorb the Cd *in situ* in the intestine and remove it from the body in the feces. This approach has been reported to be possible for aflatoxin (7). It remains to be determined whether the tested LAB can remove Cd from a food matrix or bind Cd from the digesta in the intestine. The Cd may be bound to other components or the presence of e.g. salts may interfere with absorption of Cd to the LAB. However, the results from this pilot study are promising and further investigations on the feasibility
of decontaminating liquid foods with LAB are being performed. The application of safe food grade and potentially probiotic organisms for decontamination of foods and feeds would be a significant new area for probiotic research.

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REFERENCES