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Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein

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Abstract

Heavy metals cause various health hazards. Using lactic acid bacteria (LAB), we tested the biosorption of heavy metals e.g. cadmium (Cd) (II), lead (Pb) (II), arsenic (As) (III), and mercury (Hg) (II). Cd (II) sorption was tested in 103 strains using atomic absorption spectrophotometery (AAS). *Weissella viridescens* MYU 205 (1×10^8 cells/ml) decreased Cd (II) levels in citrate buffer (pH 6.0) from one ppm to 0.459 ± 0.016 ppm, corresponding to 10.46 μg of Cd (II). After screening, 11 LAB strains were tested using various pH (pH 4.0, 5.0, 6.0, 7.0) showing the sorption was acid sensitive; and was cell concentration dependent, where the Cd (II) concentration decreased from one ppm to 0.042 (max)/0.255 (min) ppm at 1×10^{10} cells/ml. Additionally, the biosorption of Pb (II), As (III), and Hg (II) were tested using an inductively coupled plasma mass spectrometer (ICP-MS). The Hg (II) concentration was reduced the most followed by Pb (II) and As (III). Many of the bacterial cell surface proteins of *W. viridescens* MYU 205 showed binding to Hg (II) using the Hg (II) column assay. Having a CXXC motif, a ~14 kDa protein may be one of the Hg (II) binding proteins. LAB biosorption may aid the detoxification of people exposed to heavy metals.

Keywords: Heavy metal; Biosorption; Lactic acid bacteria; Detoxification; Cadmium; Mercury

1. Introduction

Heavy metals cause various health hazards. Food contaminated with heavy metals may have detrimental effects on human and animal health even at low concentrations because of gradual accumulation. The four major pollution-caused illnesses in Japan are Itai–itai disease, Minamata disease, Niigata Minamata disease, and Yokkaichi asthma. Itai–itai disease and the two Minamata diseases are caused by the heavy metals, Cd and methylmercury, respectively. Presently, heavy metal contamination is especially serious in developing countries where accumulation in the human is a concern. Therefore, Cd, Pb, As, and Hg are specified by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan as required harm factors for risk management.

LAB are common microbes used as probiotics. Probiotics show many beneficial effects, e.g. managing lactose intolerance (Savaiano and Kotz, 1989), lowering cholesterol (Danielson et al., 1989), improving immune function (Perdigón et al., 2002), preventing colon cancer (Lim et al., 2002), and the inhibiting the adherence of some pathogens (Chen et al., 2007; Mack et al., 1999; Varma et al., 2010). Probiotics such as *Lactobacillus* and *Bifidobacterium* can bind cholesterol on their cell surface, allowing efficient fecal discharge outside of the body (Gilliland et al., 1985; Tahri et al., 1996; Usman and Hosono, 1999).

Many reports show the biosorption of heavy metals by bacteria and fungi. For example, Rehman et al. (2010) reported *Candida tropicalis* CBL-1 removed Cd (II) from wastewater. Sochor et al. (2011) reported *Staphylococcus aureus* bound Cd

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(II) and may be used for the sensing of Cd (II). Lin et al. reported the biosorption of Au (III) (Lin et al., 2011) and Pt (IV) (Lin et al., 2009) in *Bacillus megatherium* D01. Li et al. (2011) reported *Bacillus cereus* showed resistance and biosorption of Ag (I). However, these species are unsuitable for food use. Conversely, people eat many LAB strains with food, especially fermented products, in which LAB are generally recognized as safe (GRAS) (Feord, 2002; Stiles and Holzapfel, 1997). We believe LAB can prevent the absorption of heavy metals into the body and oral ingestion of LAB can remove heavy metals from the body efficiently during defecation. Some reports show heavy metal biosorption by LAB (Bhakta et al., 2012; Ibrahim et al., 2006; Lin et al., 2005; Schut et al., 2011). However, further research is needed concerning the heavy metal binding capability of LAB as a probiotic for human health. Therefore, we examined the Cd (II) biosorption ability of bacteria derived from food using an *in vitro* mass-screening; and characterized other properties of selected LAB, e.g., pH effect, dose-dependence, and biosorption abilities for Pb (II), As (III), and Hg (II). Finally, we examined one of the mechanisms for Hg (II) biosorption and detoxification.

2. Materials and methods

2.1. Bacterial strains and culture conditions

One hundred three bacteria isolates from various foods, bovine and porcine intestines (called Horumon in Japan), Japanese pickles, Japanese Amazake, kimchee, and yogurt were used for bacterial samples including 50 *Lactobacillus* strains, 19 unidentified LAB derived from yogurt, eight *Weissella*, four strains of *Pediococcus*, two of *Streptococcus,* two *Enterococcus*, and 18 strains of unidentified bacteria (may be including non-LAB strains) to apply to fermented food in the future. Bacterial strains were propagated twice at 37 °C for 18 h in MRS broth with 2% (v/v) inoculum before the experiments.

2.2. Cd (II) biosorption assay

Cd (II) standard 1000 ppm solution (Cd $(NO_3)_2$ in 0.1 M HNO_3) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was diluted with 10 mM sodium citrate buffer (pH 6.0) and used as a one ppm Cd (II) solution.

The 103 bacterial strains were cultured at 37 °C for 18 h in MRS broth (Difco Laboratories, Detroit, MI) using 2% (v/v) inoculum. Bacterial cells after culture were washed three times with sterile distilled water. After washing, the pellets were suspended in 20 ml of one ppm Cd (II) solution (pH 6.0) adjusted to 1.0×10^8 cells/ml and incubated at 37 °C for 1 h. After incubation, the bacterial cells were removed using a 0.2 μm Minisart syringe filter (Sartorius Stedim Biotech GmbH, Germany); and the Cd (II) concentration of the filtrate was measured using an AAS SpectrAA-55 (Agilent Technologies, Santa Clara, CA). Buffer was used instead of bacteria in the negative control (NC).

For Cd (II) biosorption assays using various pH conditions, 10 ml of 10 mM citrate-phosphate buffer was used at pH 4.0, 5.0, 6.0, and 7.0 instead of sodium citrate buffer.

The heat-treatment test was performed at pH 5.0 and 7.0 with *Weissella viridescens* MYU 205 and *Lactobacillus mucosae* MYU 224. The bacteria were washed three times with sterile distilled water, adjusted to 1.0×10^8 cells/ml, and suspended in 10 ml of one ppm Cd (II) solution (pH 5.0 or 7.0). The bacterial suspensions were treated at 100 °C or room temperature for 1 h, and then incubated at 37 °C for 1 h. After incubation, the bacterial cells were removed using a 0.2 μm Minisart syringe filter (Sartorius Stedim Biotech GmbH); and the Cd (II) concentration of the filtrate was measured using AAS.

For Cd (II) biosorption assays at various bacterial concentrations, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} bacteria cells/ml in one ppm Cd (II) solution with 10 ml of 10 mM sodium citrate buffer (pH 6.0) was used.

2.3. Pb (II), As (III), and Hg (II) biosorption assay

Each 1000 ppm standard solution: Pb (II) (Pb $(NO_3)_2$ in 0.1 M HNO_3) (Wako Pure Chemical Industries, Ltd., Osaka, Japan), As (III) (As_2O_3 and sodium chloride (0.05%) in 1M HCl solution) (Kanto Chemical Co., Inc., Saitama, Japan), and Hg (II) ($HgCl_2$ in 0.1 M HNO_3) (Kanto Chemical Co., Inc., Saitama, Japan) was diluted with 10 mM sodium citrate buffer (pH 6.0) and used as one ppm Pb (II), As (III), or Hg (II) solution, respectively.

Pb (II), As (III), and Hg (II) biosorption assays in 10 ml of one ppm solution were performed using the same methods as the Cd (II) biosorption assays. The filtrate after biosorption was diluted 20 times with 2% nitric acid (HNO_3) solution and the concentration of the diluted solution was measured using ICP-MS ELAN DRC-e (Perkin Elmer SCIEX, Boston, MA). The concentration of the undiluted solution was evaluated 20 times compared to the concentration of a diluted solution. Buffer was used instead of bacteria in the NC.

2.4. Examination of the influence of the heavy metals on cell growth

The influence of the heavy metals on cell growth of *W. viridescens* MYU 205, *Lactobacillus sakei* MYU 10, and *L. mucosae* MYU 224 was investigated. The strains were propagated in MRS broth containing one ppm Cd (II), Pd (II), As (III), or Hg (II) with 2% (v/v) inoculum, and the pH and optical density at 600 nm (OD_{600}) of these culture media were measured after 0, 4, 8, 12, 16, 24, 36, and 48 h. MRS broth containing no heavy metal was used as a control.

2.5. Identification of Hg (II) binding proteins of W. viridescens *MYU 205*

To prepare cell wall surface proteins from *W. viridescens* MYU 205 cultured in MRS broth, the cells were washed three times with sterilized distilled water; and extracted using 2 M guanidine hydrochloride (GHCl, pH 6.0) solution at 37 °C for