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Accumulation of Aluminum to Lactic Acid Bacteria under Anaerobic Conditions

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혐기조건하 젖산균에서 알루미늄의 축적

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Abstract

Present study was investigated to evaluate the aluminum absorption effect on lactic acid bacteria(*Lactobacillus acidophilus* ATTC 4356, *Lactobacillus bulgaricus* ATTC 11842, *Lactobacillus casei* IFO 3533, and *Streptococcus thermophilus* KCTC 2185 ;LAB) and *Clostridium perfringens* ATCC 3627(CP) in artificial intestinal tract. Their growth rate, aluminum accumulation and cellular distribution was studied under anaerobic broth system. All of above microbes were inhibited by adding 10 to 100 ppm of aluminum. The degree of aluminum in LAB(*Lactobacillus acidophilus* ATCC 4356, *Lactobacillus bulgaricus* ATCC 11842, *Lactobacillus casei* IFO 3533, and *Streptococcus thermophilus* KCTC 2185) was higher than of CP. The largest amount of aluminum was accumulated in *Lactobacillus bulgaricus* ATCC 11842. Aluminum accumulation in LAB was distributed in 49.1% at cell wall, 27.3% at plasma membrane, and 23.6% at cytoplasm, respectively. This study suggests that LAB might help to eliminate the ingested aluminum in intestinal tract.

Key words : lactic acid bacteria, anaerobic broth system, aluminum, aluminum accumulation, cellular distribution.

INTRODUCTION

Aluminum is the most abundant metal, the third common element, and composes approximately 8% of crust of the earth. Human is continuously exposed to this element because of its ubiquitousness[1]. For many years, aluminum was thought to be nontoxic and largely unabsorbed from the gastrointestinal tract. However, recent date have raised serious questions about the hazards of this element[2]. Some of these hazards include diseases such as dialysis encephalopathy, osteomalacia, and anemia[3~5].

Lactic acid bacteria(LAB) influence beneficially the gastrointestinal tract of humans[6]. Some of these benefits include inhibition of pathogens, reduction in serum cholesterol, and reduction of the rick of colon cancer.

Generally, bacteria have a high surface area-to-volume ratio at a strictly physical cellular interface, and should have a high capacity for sorbing and accumulating metals[7]. Shin *et al*[8] reported that metal ions were bound easily with negative materials in cell wall of bacteria and transferred to cytoplasm by an active transport system. Therefore aluminum orally ingested into intestine may be accumulated in intestinal LAB and excreted with feces.

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The objectives of this study were to evaluate the aluminum tolerance, aluminum accumulation and cellular distribution of aluminum of LAB in anaerobic broth system.

MATERIALS & METHODS

1. Strains and media

The following five organisms were investigated in this study ; *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus bulgaricus* ATCC 11842, *Lactobacillus casei* IFO 3533, *Streptcoccus thermophilus* KCTC 2185, and *Clostridium perfringens* ATCC 3627. Between experiments, these strains were maintained frozen (−70℃) with 20% (v/v) sterile glycerol in 1ml aliquots in sterile vials. Working cultures were made by transferring the content of thawed vials to 5ml of modified EG medium and incubating anaerobically at 37℃ for 24hr.

The modified EG medium, developed by Mitsuoka[9] was composed of 2.0g of beef extract, 10.0g of proteose peptone No.3, 5.0g of yeast extract, 4.0g of sodium phosphate, 0.5g of soluble starch, 1.5g of glucose, 0.4g of L-cystein hydrochloric acid and 0.52g of tween 80 dissolved in 1000ml of distilled water and sterilized at 121℃ for 15min.

2. Effect of aluminum on the growth of lactic acid bacteria (LAB)

To evaluate the aluminum tolerance of four LAB under the anaerobic broth system, modified EG medium, bubbled with the mixed gas (CO_2: 15%, H_2: 5%, N_2: balance) to remove the dissolved oxygen, were added aluminum chloride (0, 10, 50, 100μg/ml, Sigma Chemical Co.) and inoculated with four LAB and *Clostridium perfringens* ATCC 3627(4%, v/v) as a control.

These medium were then incubated anaerobically for 48hr. at 37℃ and the samples were taken at 6hr. intervals. The cell growth was determined by measuring the optical density of the cultures at 620nm (Spectrophotometer, Varian DMS 200).

3. Accumulation of aluminum in LAB

In order to determinate the aluminum accumulation of four LAB(*Lactobacillus acidophilus* ATCC 4356, *Lactobacillus bulgaricus* ATCC 11842, *Lactobacillus casei* IFO 3533, *Streptcoccus thermophilus* KCTC 2185) and *Clostridium perfringens* ATCC 3627, these strains were incubated anaerobically in modified EG medium containing 10ppm of aluminum for 24hr. at 37℃ and the cells were harvested by centrifugation (5000×g, 30min, 4℃). The harvested cell was washed three times with 0.01M tris-HCl buffer (pH 7.0). After then, a solvent mixture of HNO_3 and HCl_4(2:1, v/v) was then added to washed cells so that cell decomposition occurred. Aluminum in decomposed samples were analyzed by atomic absorption spectrophotometer (AAS, Varian Spectra AA 30/40) and its analytical conditions were as follows : lamp current, 3.5mA ; wave length, 228.8nm ; spectral band pass, 0.5nm ; optimum working range, 0.5~2.0μg/ml ; fuel, acetylene ; support gas, air ; flame stoichiometry, oxidizing.

4. Distribution of aluminum in *Lactobacillus acidophilus* ATCC 4356 cell

To investigate the distribution of aluminum in *Lactobacillus acidophilus* ATCC 4356 cell, the strain was incubated anaerobically in modified EG medium containing 10ppm of aluminum for 24hr at 37℃ and the cells were then harvested and washed. The washed cells, held in an ice bath, were sonicated for 20min at a frequency of 16 kHz(Danbary LC 500) and the cell were fractionated into cytoplasm, plasma membrane and cell wall following procedures developed by Shin *et al*[8]. (Fig. 1). Aluminum in each fractions was determined on an AAS.

RESULTS & DISCUSSION

1. Effect of aluminum on the growth of LAB

To evaluate aluminum tolerance of LAB,

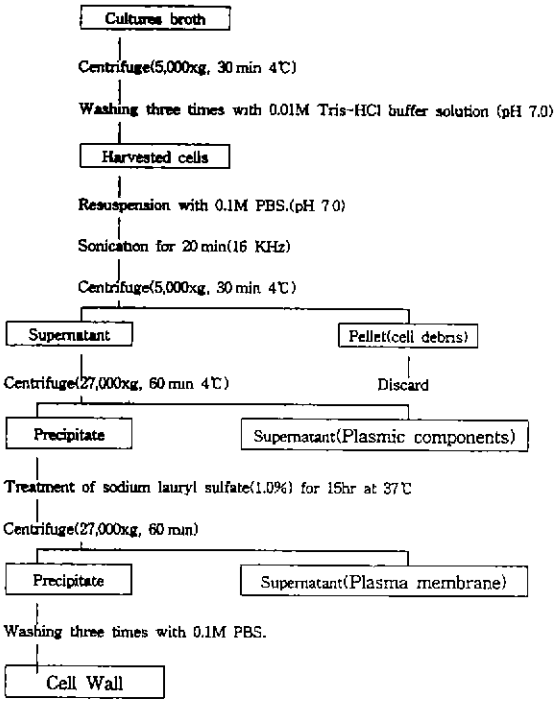

Fig. 1. Cell fractionation of *Lactobacillus acidophillus* ATCC 4356.

cell growth of four LAB and *Clostridium perfringens* ATCC 3627, intestinal pathogene was monitored in anaerobic broth system containing various concentration of aluminum (Fig. 2 to 6). All tested microbes were fairly well grown in broth containing aluminum 10μg/ml, their growth, however, were slightly inhibited at 50 and 100μg/ml of aluminum concentration. Vallee and Ulmer[10] reported that heavy metals were generally considered toxic to bacteria cells. The chemistry of bacteria cell surfaces is complex, and the potential therefore exists for heavy metal to sorb at variety of sites. Additionally, heavy metals may not only be sorbed to the surface but may also be taken up into the cytoplasm through nonspecific cation transport system[11,12]. The heavy metal, which were sorbed or taken up into cytoplasm, were thought to be toxic to bacteria cells through inhibition of enzymes, such as active transport and synthesis enzymes in plasma membrane and intracellular enzymes[8]. However, since

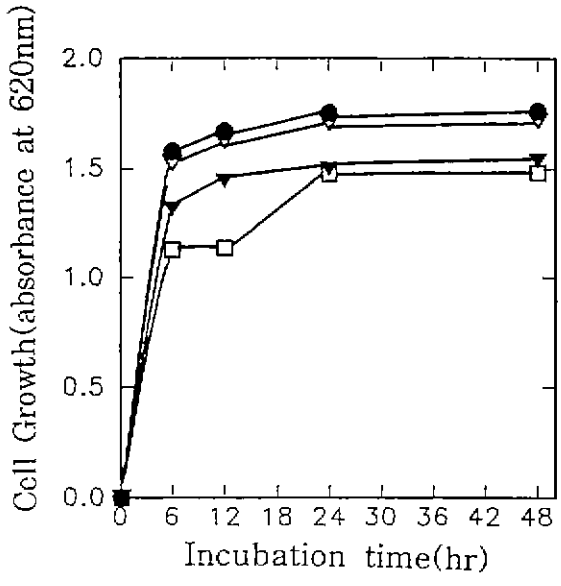

Fig. 2. Growth curve of *Clostridium perfringens* ATCC 3627 in modified EG medium containing aluminum. -●- : control, -▽- : 10 μg/ml, -▼- : 50 μg/ml, -□- : 100 μg/ml.

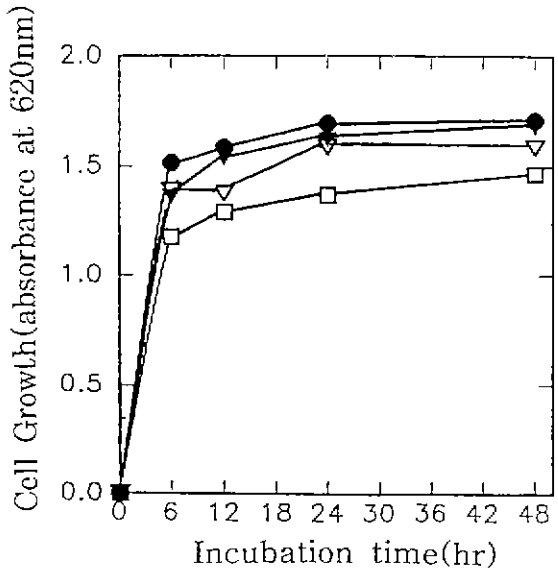

Fig. 3. Growth curve of *Lactobacillus acidophilus* ATCC 4356 in modified EG medium containing aluminum. -●- : control, -▽- : 10 μg/ml, -▼- : 50 μg/ml, -□- : 100 μg/ml.

the content of aluminum in intestinal tract was actually very small amount, and LAB were fairly well grown at 10 μg/ml of alumi-

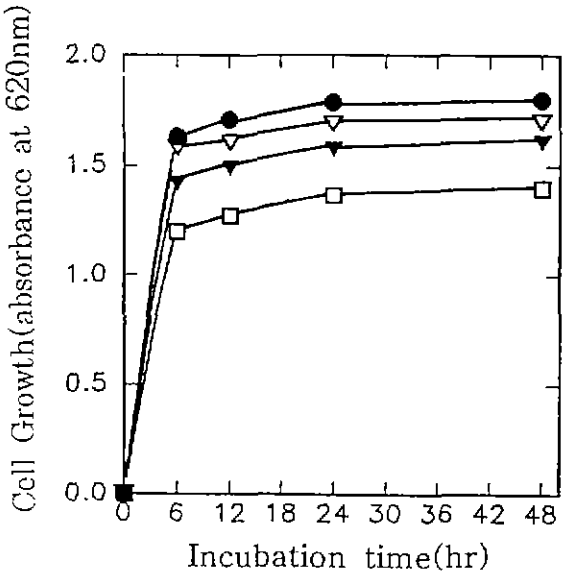

Fig. 4. Growth curve of *Lactobacillus bulgaricus* ATCC 11842 in modified EG medium containing aluminum. -●- : control, -▽- : 10 μg/ml, -▼- : 50 μg/ml, -□- : 100 μg/ml.

num in this study, LAB seemd to have a useful capacity for removing aluminum from intestinal tract.

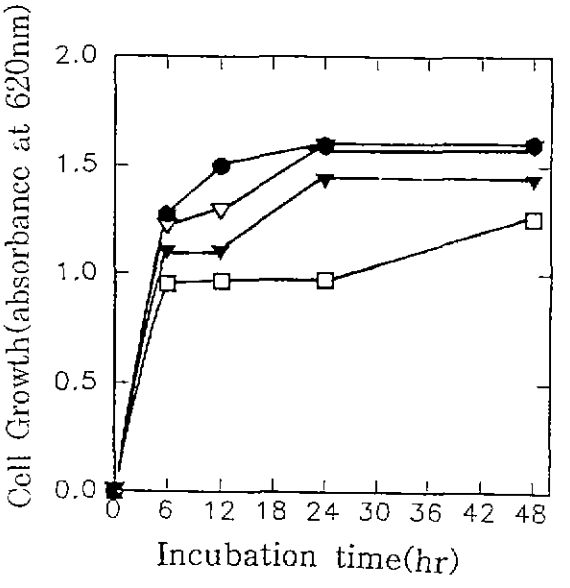

Fig. 5. Growth curve of *Lactobacillus casei* IFO 3533 in modified EG medium containing aluminum. -●- : control, -▽- : 10 μg/ml, -▼- : 50 μg/ml, -□- : 100 μg/ml.

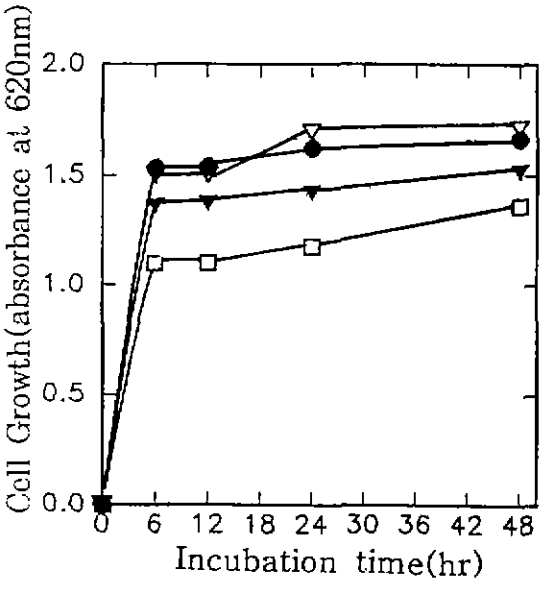

Fig. 6. Growth curve of *Streptococcus thermophilus* KCTC 2185 in modified EG medium containing aluminum. -●- : control, -▽- : 10 μg/ml, -▼- : 50 μg/ml, -□- : 100 μg/ml.

2. Accumulation of aluminum in LAB

The capacities of LAB and *Clostridium perfringens* ATCC 3627 to accumulate aluminum in the cell were compared (Table. 1) under the anaerobic broth system. The capacity of aluminum accumulation (3.52 to 4.93mg/g wet cell) of LAB was higher than that (3.08mg/g wet cell) of *Clostridium perfringens* ATCC 3627. Although tested cadmium was different, better accumulation of LAB, compared to *Clostridium perfrimgens* ATCC 3627, intestinal pathogene, has been observed earlier by Shin *et al*[8]. *Lactobacillus bulgaricus* ATCC 11842 among the LAB tested took up the largest amount of aluminum followed by *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus casei* IFO 3533 and *Streptococcus thermophilus* KCTC 2185. These results were not consistent with the previous findings of Shin *et al*[8], who reported that *Lactobacillus acidophilus* showed the largest heavy metal accumulation among the LAB tested.

3. Distribution of aluminum in *Lactobacillus acidophilus* ATCC 4356 cell

Table 1. Accumulation of aluminum in lactic acid bacteria grown in modified EG medium containing 50ppm of aluminum

Strains	Accumulated aluminum in cells (mg/g wet cell)
Clostridium perfringens ATCC 3627	3.08[a] ± 0.035[1]
Lactobacillus acidophilus ATCC 4356	4.41[b] ± 0.036
Lactobacillus bulgaricus ATCC 11842	4.93[a] ± 0.044
Lactobacillus casei IFO 3533	4.40[b] ± 0.026
Streptococcus thermophilus KCTC 2185	3.52[c] ± 0.023

[1]Mean±SD of triplications and values with different letters in the same column are signficantly different($P<0.05$).

Table 2. Distribution of aluminum in cellular structures of *Lactobacillus acidophilus* ATCC 4356

Structure	Aluminum content (mg/g wet cell)	Relative distribution(%)
Cytoplasma	1.041[b] ± 0.103[1]	23.6
Plasma membrane	1.204[b] ± 0.187	27.3
Cell wall	2.165[a] ± 0.144	49.1

[1]See foot note No. 1 of Table 2

A considerable amount (76.4%) of aluminum uptaken in *Lactobacillus acidophilus* ATCC 4356 cell was distributed in cell surface, which contained cell membrane and cell wall. Only 23.6% of aluminum was found in the cytoplasm. This was in similarity with followed observations; gram positive bacterial walls have a high affinity for metal ions because they are negatively charged at a circum-neutral pH[13,14]. These negative charged materials were composed of peptidoglycan and glycerol-based teichoic acid[15]. Heavy metals have also been shown to be transported into the interior of the bacteria cells via an energy-dependent transport system[16]. Furthermore, its transport was restricted by selective permeability of plasma membrane[8].

CONCLUSIONS

The growth of lactic acid bacteria(LAB) was not inhibited by low concentration of aluminum. LAB accumulated more aluminum than did *Clostridium perfringens* ATCC 3627, intestinal pathogene. Accordingly LAB might be useful strains to remove aluminum in intestinal tract. However, accumulation mechanism of aluminum to LAB and effect of accumulation through experimental animals needs to be studied further.

요 약

젖산균의 생육은 10μg/ml의 알루미늄이 첨가된 broth system에서 정상적으로, *Streptococcus*속보다는 *Lactobacillus*속이 더 높은 내성을 가진 것으로 나타났다. 알루미늄 50μg/ml이 첨가된 배지에서 24시간 배양시킨 젖산균에 축적된 알루미늄 양은 3.52~4.93mg/g wet cell로 3.08mg의 *Clostridium perfringens* ATCC 3627 보다 높았으며 젖산균 중 *Lactobacillus bulgalicus* ATCC 11842의 축적량이 가장 높았다. *Lactobacillus acidophilus* ATCC 4356 균체내의 알루미늄 분포상태는 세포벽에 49.1%, 원형질막에 27.3%, 세포질 23.6%로 나타났다.

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