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# Effect of Germanium-132 on the Growth of Lactic Acid Bacteria

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# 젖산균의 성장에 미치는 Ge-132의 영향

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## Abstract

The growth of lactic acid bacteria was investigated in liquid broth medium containing organic germanium compound(Ge-132, carboxyethylgermanium sesquioxide) in the range of 0.01 to 10mg/ml. Most of all lactic acid bacteria tested were tolerant and could grow better to the high Ge-132 concentration. However, the growth of *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* were inhibited in the presence of 10mg/ml Ge-132. Among 22 strains tested, lactic acid bacteria that were grown to a high degree(about 2 times) by addition of Ge-132 (10mg/ml) were *Lactococcus lactis*, *Lc. cremoris*, *Lc. diacetilactis*, *Enterococcus faecium* and *Streptococcus faecalis*. The growth of these strains were markedly accelerated in the culture medium supplemented with 1.0mg/ml Ge-132. The optimal concentration of glucose for growth of *Lc. lactis* was found to be high in medium containing Ge-132 as compared with the case of control. During cultivation, viscosity in culture broths of *Lc. lactis* and *Lc. cremoris* was rapidly elevated by adding Ge-132 to medium containing high concentration of glucose, and then decreased after incubation of long time. However, in the cultivation of *Lc. diacetilactis*, *E. faecium* and *S. faecalis*, viscosity of culture broths was not increased, even though Ge-132 was shown to be an effective stimulant of growth.

**Key words** : Ge-132, lactic acid bacteria, growth, viscosity

## Introduction

Many of metal ions are essential for microbial growth in extremely low concentration. However, they can be very toxic to microorganisms at high concentration, because of their ability to denature proteins of enzymes and cell membrane(1,2). Nevertheless, some bacteria are resistant to extremely high concentrations of single or mixtures of metals and need specific metals as growth stimulant(3).

Organic germanium compound(Ge-132, carboxyethylgermanium sesquioxide) is a novel organogermanium compound originally synthesized at Asai Germanium Research Institute of Japan, and then its chemical structure and physicochemical characteristics have already been reported(4). Ge-132 has been found to be extremely low toxicity by many toxicological and

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pharmacological studies(5,6). This compound is now being applied to the field of medicine, and then antitumor effects or antiviral activities in mouse have been reported(7-9).

Germanium uptake and bioaccumulation in some bacteria such as *Bacillus and Pseudomonas* strains by either energy-independent passive binding or an energy-dependent mechanism has also been studied(10-13). However, Changes in physiological properties of lactic acid bacteria by germanium has not been reported.

Although growth of some lactic acid bacteria has been reported to be enhanced by the presence of metals or heavy metals(3), little is known about metal-dependent exopolysaccharide production and growth of lactic acid bacteria in environments containing high levels of glucose and germanium. In another studies, germanium dioxide(GeO-2) on growth of some bacterial, yeast and algae strains was shown to be an inhibitory effect (13,14), and then bacteria generally tolerated higher concentrations of germanium than yeasts(15).

Exopolysaccharide from various mesophilic and thermophilic lactic acid bacteria strains has been already investigated(16,17). Exopolysaccharide - producing lactic acid bacteria strains are assumed to play an important role in the viscosity, texture and stabilization of fermented milk products like yoghurt and also the susceptibility of syneresis(18,19). Bacterial exopolysaccharide are classified into two groups: one, the capsular type closely associated with cell surface, and the other, the ropy or slime type loosely associated with cell surface (20-22). For example, capsular exopolysaccharide has been produced by the strains of *Streptococcus thermophilus*, *Lb. delbrueckii*, and *Lb. bulgaricus* (20,23,24). Ropy or slime exopolysaccharide has been produced by *Lc. lactis* ssp. *lactis*, *Lc. lactis* ssp. cremoris, and *Lb. casei* ssp. *casei* grown in milk and ultrafiltrate(25). However, it has been known that nature, composition and properties of microbial exopolysaccharide can be partially changed according to culture conditions (medium composition, temperature, pH), biosynthetic pathways, growth phase, and rate of microbial growth (22,26).

The present study was undertaken to identify lactic acid bacteria grown to a high degree on liquid broth medium supplemented with extremely high Ge-132 concentration, and then to survey cell growth, pH, titratable acidity and viscosity in culture broths of some lactic acid bacteria according to the concentration of Ge-132, glucose and nitrogen sources.

## Materials and Methods

### Organisms and culture media

Strains used in this study were obtained from Korea Fermented Food Research Institute(KOFRI) and National Food Research Institute(NFRI) of Japan. Lactic acid bacteria included *Enterococcus faecium*(JCM 5804), *Lb. bulgaricus*(B-56, BCH), *Lb. helveticus*(B-1, BC-10), *Lb. plantarum*(T-3), *Lc. lactis*(527, 712, SLN, SC-10), *Lc. cremoris*(H-61, HC-1, 924), *Lc. diacetilactis*(13675, N-7, DCR-1, DCR-2), *Leuconostoc mesenteroides*(LEM-1), *Pediococcus pentosaceus*(IFO 3892), *Streptococcus faecalis*(ATCC 2580) and *S. thermophilus*(510, GY). The organisms were incubated at 30℃ and maintained by daily transfer in litmus milk or MRS broth(Difco, Detroit, USA). The medium used for growth of lactic acid bacteria in this study contained glucose 10g, polypeptone 5g, yeast extract 5g and sodium succinate 5g in 1 liter deionized water(GPYS medium).

### Chemicals

Carboxyethylgermanium sesquioxide(Ge-132) was obtained from Asahi Germanium Research Institute, Tokyo, Japan. It was dissolved in deionized water containing 10M NaOH(pH 8.0) at concentration in the range of 0.1 to 100mg/ml and adjusted to pH 6.5 with 10M HCl.

### Culture

A loopful of cell from litmus milk culture broth stored in cold chamber at -70℃ was transferred to 5ml of MRS broth. The culture was grown at 30℃(except for thermophilic bacteria) for 24 hr in a tightly capped polypropylene tube(1.6×10cm). Cultures prepared were used as inocula in subsequent experiments. To identify lactic acid bacteria grown to high levels by Ge-132 addition, lactic acid bacteria was grown in 1,200$\mu\ell$ of GPYS medium, preculture broth 150$\mu\ell$ and various germanium solution 150$\mu\ell$ in a 5ml test tube. Growth was measured by reading the optical density at 655nm with a microplate reader(Biorad Co.). In the other experiments, the growth of lactic acid bacteria was performed to large test tube(2.5×15cm) containing

mixture(30ml) of the same conditions. Cell viability was determined by the plate dilution method using MRS agar plate. Serial dilution of each sample were plated in duplicate and were incubated at 30℃ for 24 hr. Results were expressed as colony forming units(logCFU/ml).

### Determination of titratable acidity and viscosity

Titratable acidity expressed as lactic acid percentage was measured with 0.1 N NaOH as standard solution. Viscosity of culture broth and culture supernatant fluid were measured by using a capillary type viscometer (Ostwald type, 0.5mm tube diameter, Vidrex, Fukuoka, Japan). The viscometer was placed in a water bath(4 0℃). The sample(5ml) was added to viscometer and kept for 10min. After adjusting the tested sample to 4 0℃, the sample was released inside the capillary tube and the time required for passage of sample between menisci was determined with stopwatch. This procedure was repeated five times for each sample. To test the another sample, the viscometer was washed and rinsed with distilled water and 99% ethanol. Viscosity expressed in centistoke(cSt) was calculated by the equation of flow time(seconds) of sample at 40℃ × viscosity of water at 40℃ / flow time(seconds) of water at 40℃.

## Results and Dscussion

### Effect of Ge-132 on the growth of lactic acid bacteria

As shown in Table 1, growth of lactic acid bacteria was promoted in the presence of Ge-132 (0.01 to 10mg/ml) on GPYS medium. Most of the lactic acid bacteria tested were tolerant to high Ge-132 concentration and exhibited growth in the presence of up to 10mg/ml Ge-132. However, in *P. pentosaceus* and *Leu. mesenteroides*, their growth was showed to be slightly inhibited at 10mg/ml Ge-132. The growth of *E. faecium*, *Lc. cremoris*, *Lc. lactis*, *Lc. diacetilactis* and *S. faecalis* at 10mg/ml Ge-132 for 12 hrs was about 2 times than that observed in the absence of Ge-132. In addition, Most of 5 general bacterial, 12 yeast and 3 mold strains tested were tolerant to 1mg/ml Ge-132 and their growth were slightly inhibited at 10mg/ml Ge-132 (data not shown).

Table 1. Effect of Ge-132 on growth(OD at 655nm) of lactic acid bacteria

| Lactic acid bacteria | Concentration of Ge-132 (mg/ml) | | | |
|---|---|---|---|---|
| | 0 | 0.1 | 1 | 10 |
| *P. pentosaceus* IFO 3892 | 0.975 | 0.964 | 0.962 | 0.822 |
| *E. faecium* JCM 5804 | 0.476 | 0.479 | 0.555 | 0.816 |
| *Lb. bulgaricus* B-56 | 0.367 | 0.372 | 0.384 | 0.395 |
| *Lb. bulgaricus* BCH | 0.329 | 0.337 | 0.348 | 0.352 |
| *Lb. helveticus* B-1 | 0.649 | 0.642 | 0.725 | 0.725 |
| *Lb. helveticus* BC-10 | 0.986 | 1.007 | 1.036 | 1.163 |
| *Lb. plantarum* T-3 | 1.098 | 1.103 | 1.149 | 1.135 |
| *Lc. cremoris* H-61 | 0.140 | 0.140 | 0.245 | 0.276 |
| *Lc. cremoris* HC-1 | 0.444 | 0.446 | 0.513 | 0.760 |
| *Lc. cremoris* 924 | 0.428 | 0.430 | 0.510 | 0.778 |
| *Lc. lactis* 527 | 0.301 | 0.317 | 0.399 | 0.794 |
| *Lc. lactis* 712 | 0.385 | 0.404 | 0.485 | 0.781 |
| *Lc. lactis* SLN | 0.350 | 0.337 | 0.396 | 0.703 |
| *Lc. lactis* SC-10 | 0.293 | 0.282 | 0.353 | 0.573 |
| *Lc. diacetilactis* 13675 | 0.458 | 0.441 | 0.498 | 0.708 |
| *Lc. diacetilactis* N-7 | 0.347 | 0.354 | 0.405 | 0.783 |
| *Lc. diacetilactis* DCR-1 | 0.388 | 0.403 | 0.435 | 0.723 |
| *Lc. diacetilactis* DCR-2 | 0.348 | 0.358 | 0.400 | 0.727 |
| *Leu. mesenteroides* LEM-1 | 0.437 | 0.445 | 0.498 | 0.253 |
| *S. faecalis* ATCC 2580 | 0.366 | 0.355 | 0.543 | 0.784 |
| *S. thermophilus* 510 | 0.221 | 0.217 | 0.234 | 0.302 |
| *S. thermophilus* GY | 0.232 | 0.235 | 0.238 | 0.313 |

Lactic acid bacteria were cultivated at 30℃ for 12 hr on GPYS medium containing Ge-132 in the range of 0.1 to 10mg/ml. Growth was measured by reading the optical density at 655nm with a microplate reader (Biorad Co.). Each value represents the average of duplicate measurements.

Various metals such as Mg, Mn, Fe, Ca, K, and Na has been reported to be enhanced in growth of lactic acid bacteria under specific culture medium. Moreover, most of macronutrient minerals as well as trace elements were needed in low concentration for growth stimulation of lactic acid bacteria(3). Nevertheless, most of all lactic acid bacteria tested in this studies could tolerate and grow better at high Ge-132 concentration (10mg/ml), suggesting that Ge-132 has the existence of very efficient adaptive mechanisms. The growth in *E. faecium*, *Lc. cremoris*, *Lc. lactis*, *Lc. diacetilactis* and *S. faecalis* grown at 10mg/ml Ge-132 for 12 hr was about 2 times than that observed in the absence of Ge-132 but some lactic acid bacteria such as *P. pentosaceus* and *Leu. mesenteroides* was showed to be slightly inhibited by the extremely high Ge-132 concentration. In case of germanium dioxide(GeO-2), the growth of some bacterial, yeast and algae strains was shown to be inhibitory(13-15).

## Changes in viable cell growth, titratable acidity and viscosity of culture broth

Changes in viable cell growth (expressed as colony-forming unit), titratable acidity (lactic acid percentage) and viscosity in culture broths of *Lc. lactis* 712, *Lc. cremoris* 924 and *E. faecium* JCM 5804 incubated at 30℃ for 48 hr was shown in Table 2. Viable cell growth and titratable acidity of 3 strains examined were markedly promoted after incubation of 8 hr in the presence of 10mg/ml Ge-132. The titratable acidity of culture broths was increased with corresponding increase in growth of lactic acid bacteria. Viscosity in culture broth of *Lc. lactis* 712 and *Lc. cremoris* 924 were rapidly increased after incubation of 12 hr, but decreased after 24 hr. The maximum viscosity for both culture broths was during the late stationary phase of growth. In contrast to above 2 strains, during incubation of *E. faecium* JCM 5804, increased changes of viscosity was not clearly appeared, even though the growth was promoted by Ge-132.

Table 2. Changes in viable cell growth, titratable acidity and viscosity in culture broth of *L. lactis* 712, *L. cremoris* 924 and *E. faecium* JCM 5804

| Cultivation time(hr) | Lc. lactis 712 | | | Lc. cremoris 924 | | | E. faecium JCM 5804 | | |
|---|---|---|---|---|---|---|---|---|---|
| | CFU | TA | VC | CFU | TA | VC | CFU | TA | VC |
| 0 | 0.32 | 0.04 | 0.78 | 0.28 | 0.03 | 0.72 | 0.15 | 0.03 | 0.74 |
| 4 | 1.34 | 0.18 | 0.82 | 2.36 | 0.14 | 0.79 | 2.25 | 0.05 | 0.74 |
| 8 | 6.64 | 0.37 | 0.94 | 3.39 | 0.21 | 0.83 | 3.39 | 0.12 | 0.75 |
| 12 | 6.78 | 0.47 | 1.38 | 6.21 | 0.33 | 0.87 | 5.57 | 0.17 | 0.77 |
| 16 | 6.43 | 0.53 | 1.57 | 6.87 | 0.42 | 1.12 | 6.62 | 0.25 | 0.78 |
| 20 | 6.26 | 0.54 | 1.48 | 6.32 | 0.44 | 1.35 | 6.80 | 0.39 | 0.78 |
| 24 | 5.82 | 0.58 | 1.39 | 5.86 | 0.47 | 1.30 | 7.12 | 0.44 | 0.79 |
| 36 | 5.73 | 0.56 | 1.15 | 5.64 | 0.52 | 1.12 | 6.81 | 0.45 | 0.80 |
| 48 | 5.64 | 0.62 | 0.95 | 5.22 | 0.49 | 0.98 | 6.43 | 0.43 | 0.79 |

Lactic acid bacteria were cultivated at 30℃ for 48 hr in GPYS medium containing 10mg/ml Ge-132. Refer to text on viable cell growth(expressed as CFU), titratable acidity and viscosity. Each value represents the average of duplicate measurements.
Abbreviations : CFU, log CFU(colony-forming unit)/ml : TA, titratable acidity(lactic acid percentage) : VC, viscosity(cSt).

*Lc. cremoris* and *Lc. lactis* was shown to increase viscosity of culture broth on medium containing a high Ge-132 concentration during cultivation, while viscosity in *Lc. diacetilactis* and *S. faecalis* as well as *E. faecium* (Table 2) was very low(data not shown), even though growth of 3 strains were rapidly increased. These effect of germanium for both stimulation of growth and increase of viscosity was found to be strain-dependent on GPYS medium. In addition, we guess that decrease of viscosity in culture broth after incubation of long time was due to the presence of lactic acid and glycohydrolase capable of degrading the polymer(25,27,28).

## Time course of the growth of lactic acid bacteria according to Ge-132 concentration

During the cultivation at 30℃ for 24 hr, the growth of *Lc. lactis* 712 and *E. faecium* JCM 5804 was investigated on GPYS medium at a concentration of Ge-132 in the range of 0.01 to 10mg/ml. As shown in Fig. 1, the growth of *Lc. lactis* 712 was accelerated after cultivation of 4 hr and appeared to maximum state at various Ge-132 concentration after cultivation from 8 to 12 hr. No differences occurred among the concentration from 0.01 to 0.1mg/ml Ge-132 in growth rate of *Lc. lactis* 712, but the growth of this strain was markedly promoted at 10mg/ml Ge-132. In *E. faecium* JCM 5804, the growth was increased to incubation of 24 hr, the growth patterns were similar with *Lc. lactis* 712 at all Ge-132 concentration tested and difference of growth rate by Ge-132 addition was lower than that of *Lc. lactic* 712.

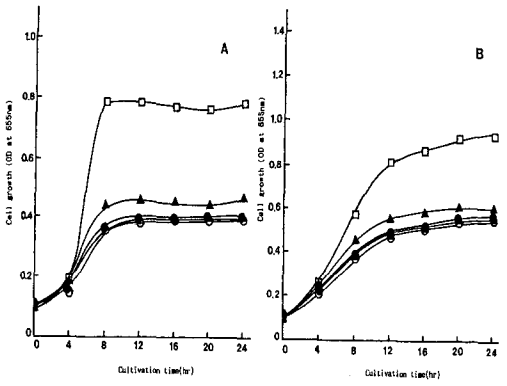


Fig. 1. Effect of germanium concentration on the growth of *Lactococcus lactis* 712 (A) and *Enterococcus faecium* JCM 5804 (B) in GPYS medium.
○: 0 mg/ml, ●: 0.01 mg/ml, △: 0.1 mg/ml, ▲: 1 mg/ml, □: 10mg/ml.

## Relationship of Ge-132 and glucose concentration on the growth of lactic acid bacteria

The effect of Ge-132 on the growth of lactic acid

bacteria under various glucose concentration is investigated when grown on GPYS medium in the presence and absence of 10mg/ml Ge-132. As typically shown in Fig. 2, when grown at 30℃ for 12 hr on GPYS medium in the absence of Ge-132, the growth of Lc. lactis 712 was gradually increased to 0.4%(w/v) glucose and decreased thereafter. Moreover, in medium of Ge-132 addition(10mg/ml), *Lc. lactis* 712 was able to grow better to 1%(w/v) glucose concentration being optimal concentration of glucose and the growth was significantly accelerated to above 2 times as compared with the case of control. In *E. faecium* JCM 5804, optimal concentration of glucose for growth was shown to be 0.6% in presence and absence of Ge-132. the growth patterns were similar with *Lc. lactis* 712 at all glucose concentration tested.

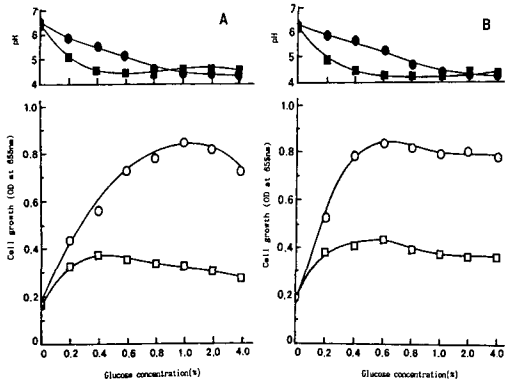


Fig. 2. Effect of glucose concentration on the growth and pH of *Lactococcus lactis* 712 (A) and *Enterococcus faecium* JCM 5804 (B) in GPYS medium with or without 10mg/ml Ge-132.
○, ●: with germanium : □, ■: without germanium.

On the other hand, changes in pH of culture broths with Ge-132 were similar between the two strains tested and pH was slowly decreased to 0.8% glucose. However, in absence of Ge-132, pH was rapidly decreased at low glucose concentration. pH value in broth culture with or without addition of Ge-132 was similar in the concentration of over 0.8 to 1.0% glucose.

## Relationship of Ge-132 and nitrogen concentration on the growth of lactic acid bacteria

To test effect of Ge-132 on the growth of lactic acid bacteria according to nitrogen sources concentration, *Lc. lactis* 712 and *E. faecium* JCM 5804 were incubated at 30℃ for 12 hr on GPYS medium in the presence and absence of 10mg/ml Ge-132. As shown in Fig. 3, the growth of lactic acid bacteria were rapidly increased by the range of 0.6 to 0.8%(w/v) nitrogen sources on GPYS medium with Ge-132. However, in presence of Ge-132, optimal concentration of nitrogen sources for growth of Lc. lactis 712 and *E. faecium* JCM 5804 were 0.4 and 0.6%, respectively. In particular, the growth rate of *Lc. lactis* 712 and *E. faecium* JCM 5804 at GPYS medium supplemented with 10mg/ml Ge-132 were significantly accelerated by increasing the concentration of nitrogen sources to above 2 or 3 times as compared with the case of control. On the other hand, changes in pH values of culture broth of 2 strains tested were a similar trend at up to 1% nitrogen sources, but in low concentration of nitrogen sources as well as glucose, pH values of culture broth without Ge-132 were more rapidly decreased than those of culture broth with Ge-132.

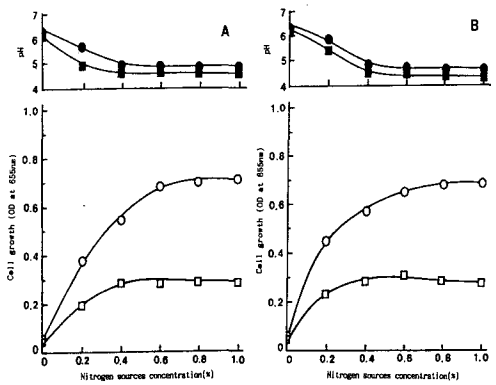


Fig. 3. Effect of nitrogen sources concentration on the growth and pH of *Lactococcus lactis* 712 (A) and *Enterococcus faecium* JCM 5804 (B) in GPYS medium with or without 10mg/ml Ge-132.
○, ●: with germanium : □, ■: without germanium.

## Changes in viscosity of culture broth according to glucose concentration

To assess the nature of viscous material produced by the liquid cultivation of *Lc. lactis* 712, viscosity of culture broth and supernatant fluid were assessed according to glucose concentration. As shown in Fig. 4, viscosity of culture broth from *Lc. lactis* 712 grown GPYS medium supplemented with 10mg/ml Ge-132 was

rapidly elevated with increasing concentration of glucose to medium up to 1% glucose, and gradually decreased due to inhibition of growth of *Lc. lactis* 712 at over 1% glucose. Viscosity of culture supernatant fluid in presence of Ge-132 was gradually increased to 10% glucose, and then exhibited significantly lower than that of culture broth when grown on GPYS medium of 1% glucose. In contrast to the result of Ge-132 addition, changes in viscosity of culture broth and supernatant fluid without Ge-132 to the medium were not nearly appeared to 1% glucose. After this point, although cell growth was gradually decreased, viscosity was reversely increased. This might be due to the higher concentration of glucose.

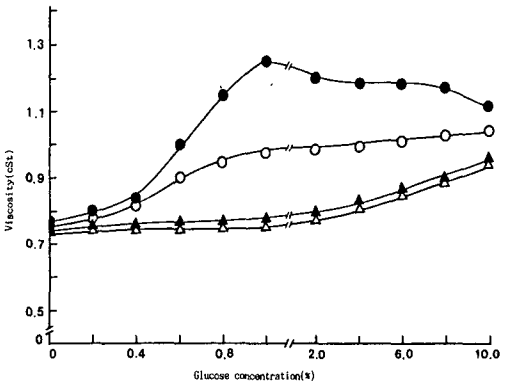


Fig. 4. Changes in viscosity of culture broth and supernatant fluid from *Lactococcus lactis* 712. Viscosity was measured by using Ostwald viscometer with 4ml of solution at 40℃. The strain was cultivated at 30℃ for 12 hr in GPYS medium containing 10mg/ml Ge-132 and glucose concentration from 0.2 to 10%.
● : culture broth with germanium.
○ : supernatant fluid with germanium.
▲ : culture broth without germanium.
△ : supernatant fluid without germanium.

Bacteria synthesize a number of polysaccharide which are defined by their location relative to the cell. Bacterial polysaccharide are mainly located in the cytosol (as carbon sources), cell wall constituents(peptidoglycan, teichoic acid) and outside cell wall(17). The exopolysaccharide production and capsular structure in the bacteria may contribute to uptake of metal ions(29). The growth and EPS production of lactic acid bacteria was favoured by presence of macro-elements such as Mg, Mn and Fe metal(30). In this studies, *Lc. lactis* and *Lc. cremoris* cultured in GPYS medium supplemented with Ge-132 has a free and cell wall-attached exopolysaccharide regarded as ropy type, since viscosity in both suspension of centrifugal harvested cell and supernatant fluid of lactic acid bacteria were appeared. However, both capsular and unattached polysaccharide are produced by the same organism, and then distinguishing between the two forms can be difficult(17).

In case of *Lc. lactis* and *Lc. cremoris*, it was also possible that the production of viscous material may be associated with carbohydrate metabolism, where viscosity of culture broth was increased with addition of glucose and Ge-132. Recently, the viscous materials of both cell surface and supernatant fluid was confirmed to be polysaccharide by gas chromatographic analysis(data not shown). We are now investigating both Ge-132 uptake into an inner cell and the sugar composition of exopolysaccharide produced during growth of some lactic acid bacteria.

It is interesting to note that viscosity in culture broth of lactic acid bacteria was increased by adding Ge-132 to the medium containing high concentration of glucose. In particular, viscosities of the culture broths were much higher in comparison to the supernatant fluid of culture broths. Consequently, we found that Ge-132 was an activator to promote the growth and production of cell free or bounding viscous exopolysaccharide in some lactic acid bacteria, suggesting that this compound was related to sugar metabolism.

## 요 약

유기게르마늄(Ge-132, carboxyethylgermanium)에 의한 22가지 젖산균의 성장 효과를 0.01~10mg/ml의 농도로 첨가된 GPYS 액체배지에서 조사하였다. 시험한 대부분의 젖산균은 고농도의 게르마늄에서도 내성이 있었고, 게르마늄의 농도가 높을수록 성장을 더욱 촉진시키는 효과가 나타났다. 게르마늄이 10mg/ml의 농도로 첨가된 GPYS배지에서 *Lactococcus lactis*, *Lc. cremoris*, *Lc. diacetilactis*, *Enterococcus faecium* 및 *Streptococcus faecalis*는 2배 이상 생육촉진의 효과를 나타내었으나, *Leuconostoc mesenteroides*와 *Pediococcus pentosaceus*는 저해를 나타내었다. *Lc. lactis*와 *Lc. cremoris*의 경우, 배양액의 점도는 게르마늄이 첨가된 GPYS배지에서 급격히 증가되었지만, 장시간 배양에 의해서는 약간 감소되었다. 그러나 *Lc. diacetilactis*, *E.*

*faecium*와 *S. faecalis*의 경우, 게르마늄의 첨가에 의하여 생육은 현저하게 촉진되었지만, 배양중의 점도는 증가되지 않았다.

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## References

1. Gadd, G.M. and Griffiths, A.J. (1978) Microorganisms and heavy metal toxicity. *Microb. Ecol.*, 4, 303-317
2. Trevors, J.T., Oddie, K.M. and Belliveau, B.H. (1985) Metal resistance in bacteria. *FEMS Microbiol. Rev.*, 32, 39-54
3. Boyavel, P. (1989) Lactic acid bacteria and metal ions. *Lait*, 69, 87-113
4. Tsutsui, M., Kakimoto, N. and Darrell, D.A. (1981) Crystal structure of "carboxyethylgermanium sesquioxide". *J. Am. Chem. Soc.*, 98, 8287-8289
5. Nagata, T., Nagata, T., Aramaki, Y., Enomoto, M., Isaka, H. and Otsuka, J. (1978) Chronic intravenous toxicity study with carboxyethylgermanium sesquioxide in beagle dogs. *Pharmacometrics*, 16, 671-682
6. Tomizawa, S., Suguro, N. and Nagashima, M. (1978) Studies on general pharmacological effects of some germanium compounds. *Pharmacometrics*, 16, 671-682
7. Satoh, H. and Iwaguchi, T. (1979) Antitumor activity of new novel organogermanium compound, Ge-132. *Cancer and Chemother.*, 6, 79-83
8. Aso, H., Suzuki, F., Yamaguchi, T., Hayashi, Y., Ebina, T. and Ishida, N. (1985) Induction of interferon and activation of NK cells and macrophages in mice by oral administration of Ge-132, an organic germanium compound. *Microbiol. Immunol.*, 29, 65-74
9. Ishikawa, M., Aoki, T., Takayanagi, Y. and Sasaki, K. (1989) The effects of carboxyethylgermanium (Ge-132), an immunomodulator, on nociception in mice. *Res. Commun. Chem. Pathol. Pharmacol.*, 66, 481-484
10. Chmielowski, J. and Klapcinska, B. (1986) Bioaccumulation of germanium by Pseudomonas putida in presence of two selected substrate. *Appl. Environ. Microbiol.*, 51, 1099-1103
11. Van-Dyke, M.I., Lee, I. and Trevors, J.T. (1989) Germanium accumulation by bacteria. *Arc. Bicrobiol.*, 152, 533-538
12. Van-Dyke, M.I., Parker, W., Lee, H. and Trevors, J.T. (1990) Germanium accumulation by Pseudomonas stutzeri AG259. *Appl. Microbiol. Biotech.*, 33, 716-720
13. Slawson, R.M., Van-Dyke, M.I., Lee, H. and Trevors, J.T. (1992) Germanium and silver resistance, accumulation, and toxicity in microorganisms. *Plasmid*, 27, 72-79
14. Tatewaki, M. and Mitzuno, M. (1979) Growth inhibition by germanium dioxide in various algae, especially in brown algae. *Jap. J. Phyco.*, 24, 205-212
15. Van-Dyke, M.I., Lee, H. and Trevors, J.T. (1989) Germanium toxicity in selected bacterial and yeast strains. *J. Ind. Microbiol.*, 4, 299-306
16. Cerning, J. (1990) Exocellular polysaccharide produced by lactic acid bacteria. *FEMS Microbiol. Rev.*, 87, 113-130
17. Cerning, J. (1995) Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Lait*, 75, 463-472
18. Schellhaass S.M. (1983) Characterization of exocellular slime produced by bacterial starter cultures used in the manufacture of fermented dairy products. PhD Thesis, University of Minnesota, St. Paul
19. Wacher-Rodarte, C., Calvan, M.V., Farres, A., Gallardo, F., Marshall, V.M.E. and Garcia-Garibay, M. (1993) Yoghurt production from reconstituted skim milk powders using different polymer and nonpolymer forming starter cultures. *J. Dairy Res.*, 60, 247-254
20. Ariga, H., Urashima, T., Michihala, E.,Ito, M., Morizono, N., Kimura, T. and Takahashi, S. (1992) Extracellular polysaccharide from encapsulated *Streptococcus salivarius* ssp. *thermophilus* OR 901 isolated from commercial yogurt. *J. Food Sci.*, 57, 625-628
21. Hess, S.J. (1994) Rheology of yogurt manufactured using exopolysaccharide-producing strains of *Lactobacillus delbrueckii* ssp. bulgaricus. MS Dissertation, Pennsylvania State Univ., Pennsylvania
22. Vuyst, L.D. and Degeest, B. (1999) Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol. Rev.*,

23, 153-177

23. Hassan, A.N., Frank, J.F., Farmer, M.A., Schmidt, K.A. and Shalabi, S.I. (1995) Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. *J. Dairy Sci.*, 78, 2624-2628
24. Adapa, S. and Schmidt, K.A. (1998) Physical properties of low-fat sour cream containing exopolysaccharide-producing lactic acid bacteria. *J. Food Sci.*, 63, 901-903
25. Cerning, J., Bouillanne, C., Landon, M. and Desmazuaud, M. (1992) Isolation and characterization of exopolysaccharide from slime-forming lactic acid bacteria. *J. Dairy Sci.*, 75, 692-699
26. Rimpilainen, M., Valkonen, K.H. and Forsen, R. (1986) Isolation and characterization of the plasma membrane from slime-forming, encapsulated *Streptococcus cremoris*. *Int. J. Biochem.*, 18, 93-98
27. Macura, D. and Townsley, P.M. (1984) Scandinavian ropy milk - Identification and characterization of endogenous ropy lactic streptococci and their extracellular secretion. *J. Dairy Res.*, 67, 735-744
28. Cerning, J., Bouillanne, M., Desmazeaud, M. and Landon, M. (1988) Exocellular polysaccharide production by *Streptococcus thermophilus*. *Biotechnol. Lett.*, 10, 255-260
29. Cerning, J., Bouillanne, C., Landon, M. and Desmazeaud, M.J. (1990) Comparison of exocellular polysaccharide production by thermophilic lactic acid bacteria. *Sciences des Aliments*, 10, 443-451
30. Gamar-Nourani, L., Blondeau, K. and Simonet, J.M. (1998) Influence of culture conditions on exopolysaccharide production by Lactotobacillus rhamnosus strain C83. *J. Appl. Microbiol.*, 85, 664-672

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