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Effect of Titanium-Ion on the Growth of Various Bacterial Species

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There are a number of studies that explain the metabolism and roles of metallic titanium and titanium-ion. One of the most intriguing results from these studies is the finding of metallic titanium having no bacteriostatic effects on oral bacterial species. In this research, the effects of titanium-ion on the growth of twenty-two bacterial species, some of which are commonly found in foods such as yoghurt, kimchi, and soy fermented products, were investigated. All but two bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* appeared to be sensitive to titanium-ion. These two species were grown on 360 μg/ml of titanium-ions, and they were found to be resistant to the titanium-ion. Both the wild-type and plasmid-cured *E. coli* showed good growth in a medium with 200 μg/ml of titanium-ions. These results suggest that titanium-resistance was independent from the effects of the plasmid in *E. coli*.

***Key words*:** titanium-ion, titanium-resistance, *E. coli*

Environmental contaminations by certain metal compounds are bringing about serious problems to human health, including genetic disorders. It has been reported that metal compounds such as iron (Demerec *et al*. 1951), manganese (Demerec *et al*., 1951), mercury (Gadd and Griffiths, 1978), cadmium (Gadd and Griffiths, 1978; Yu *et al*., 1986; Yu *et al*., 1990), and lead (Muro and Goyer, 1969; Rho and Kim, 2002) have a negative influence on microorganisms by affecting their growth, morphology, mutagenicity, and biochemical activities.

Titanium is widely distributed in the earth's crust where it is the eighth most common chemical element. Titanium tetrachloride is a colorless to pale yellow liquid that has fumes that give off a strong odor. It is not a naturally occurring substance in the environment but is made from minerals that contain metallic titanium. It is utilized to produce titanium metal and other titanium-containing compounds, such as titanium trichloride and titanium dioxide. Titanium dioxide is extensively used as a white pigment in paint, plastics, paper filling, and other hardware products, and also as an intermediate to produce other chemicals, such as ceramics. It is also used as a coloring agent in food industry, in cosmetics, and in iridescent glass. Also, titanium dioxide, among with several other titanium compounds, is used as a catalysts for various chemical reactions. Ferro titanium is widely used in the steel industry. Titanium exists largely in the +3 and +4 oxidation states. As a result, their ions have neither d electrons nor a closed-shell configuration which in turn precludes biochemically relevant redox chemistry. Titanium alloys are increasingly used for dental implants because of their biocompatibility (Breme, 1989; Elagli *et al*., 1989) corrosion resistance (Breme and Wadewitz, 1989; Elagli *et al*., 1991) and bio-functionality (Breme, 1989).

Metallic titanium has no bacteriostatic effect on oral bacteria of different morphology, respiratory type and sugar fermentation type (Joshi and Eley, 1988; Elagli *et al*., 1992). Titanium dioxide levels of up to 0.1 M do not affect the growth of oral bacteria, but above this 0.1 M mark, it does have some affect (Elo *et al*., 1972). Metallic titanium can selectively accumulate in the lung and adjacent lymph nodes by being exposed to a high concentration of titanium tetrachloride or by being chronically exposed to the same compound, and this accumulation can lead to pulmonary injury (Elo *et al*., 1972; Garabrant *et al*., 1987). Specifically, chronic exposure to titanium may cause the pulmonary granulomatous disease (Redline *et al*., 1986). The dinoflagellate *Gymnodinum brevis* concentrates titanium intracellularly by a factor of 8×10^4 against seawater, but no biological role for titanium has been shown (Phipps, 1976). The aim of this study, using twenty-two bacterial species, was to determine the influence of the titanium-ions which possibly is of importance in influencing microorganisms' growth and additionally influencing the ecosystems of food.

Fourteen strains of gram-positive bacteria and 8 strains of Gram-negative bacteria were used in this study. Bacterial cells were aerobically grown in flasks with LB medium (1.0% peptone, 0.5% yeast extract, 0.5% sodium chloride, pH 7.0) containing indicated titanium-ion at 37°C for 2 days, and *Lactobacillus* spp. was anaerobically grown at 25°C for 2 days. The cytotoxicity of the titanium ion concentration was measured as IC50 values (inhibitory concentration values, *i.e.* titanium-ion concentration required to inhibit via-

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bility by 50%) (Lyu *et al*., 2001), and MIC (minimum inhibitory concentration) was estimated by an agar dilution technique (Joshi and Eley, 1988). Each assay was done in triplicates. Curing of *E. coli* plasmid was carried out by treatment with mitomycin C at a concentration of 10 μg/ml as described by Chakrabarty (1972). Plasmid DNA was isolated using an alkaline lysis method (Birnboim and Doly, 1979). The plasmid DNA was separated on a 1% agarose gel with a mini-gel electrophoresis system (Mupid-2, Cosmo Bio. Co., Japan). Bacterial growth was determined by observing the optical density measured at 660 *nm* using a spectrophotometer (UV-120-02, Shimadzu Inc., Japan). The titanium-ion used titanium atomic absorption standard solution, which contains 1,020 μg/ml of Ti in water, d 1.000 (Aldrich Chemical Co., Inc., USA).

Table 1 illustrates the effects of the titanium ion on the growth of various bacterial species. *Bacillus acidocaldarius, B. circulans, B. polymyxa, Mycobacterium smegmatis, Staphylococcus faecalis, Alcaligenes faecalis, Enterobacter aerogenes,* and *Pseudomonas fluorescens* could not grow in LB medium containing 50 μg/ml of titanium ions (Table 1). *B. subtilis, Lactobacillus brevis* subsp. *brevis, L. lactis, L. oris, L. parabuchneri, Staphylococcus aureus,* and *Salmonella typhimurium* were abundantly grown in LB medium with 50 μg/ml of titanium ions, but not in medium with 60 μg/ml of titanium ions. *Staphylococcus mutans* and *Klebsiella pneumonia* illustrated a small amount of growth in 80 μg/ml of titanium ions. However, *E. coli* and *P. aeruginosa* showed a high resistance to titanium ion by being able to grow in copious amounts in a medium with up to 350 μg/ml of titanium ions. MIC of *E. coli* and *P. aeruginosa* were measured at 360 μg/ml and 350 μg/ml of titanium ions, respectively. According to these results, the titanium-ions appeared to have strong toxic effects on the tested strain with the exception of two: *E. coli* and *P. aeruginosa.*

Fig. 1 shows *E. coli* and *P. aeruginosa* cell growth in the LB medium with various concentrations of the titanium-ion. In the medium without the titanium-ion the maximum cell growth of *E. coli* was obtained after the cultivation of 12 h and thereafter, the culture was maintained at the same population of cells. In the medium with high concentrations of the titanium ion (150 μg/ml to 200 μg/ml) a lag phase of *E. coli* lasted for 3 h and then the cell growth plateaued at the maximum population level for 18 h (Fig. 1A). Furthermore, after the lag phase of *E. coli* was sustained for 9 h in the medium with 300 μg/ml of titanium-ions the growth of *E. coli* gradually

Table 1. Effects of the titanium ion on bacterial growth

Strain	MIC (μg/ml of titanium-ion)
Gram-positive	
Bacillus acidocaldarius KCTC 1825	40
Bacillus circulans KCTC 1662	20
Bacillus polymyxa KCTC 1663	20
Bacillus subtilis KCCM 12248	50
Bacillus thermoglucosidasius KCTC 1665	60
Lactobacillus brevis subsp. *brevis* KCTC 3102	50
Lactobacillus lactis KCTC 2181	50
Lactobacillus oris KCTC 3502	50
Lactobacillus parabuchneri KCTC 3503	50
Mycobacterium smegmatis KCTC 1057	20
Staphylococcus aureus KCTC 1927	50
Staphylococcus faecalis KCCM 11814	40
Staphylococcus lactis KCTC 3191	60
Staphylococcus mutans KCTC 3065	80
Gram-negative	
Alcaligenes faecalis KCTC 1004	40
Enterobacter aerogenes KCCM 12177	20
Escherichia coli KCCM 11591	360
Klebsiella pneumoniae KCTC 1560	80
Pseudomonas aeruginosa KCCM 11266	350
Pseudomonas fluorescens KCTC 1645	30
Pseudomonas stutzeri KCTC 1066	60
Salmonella typhimurium KCTC 1926	50

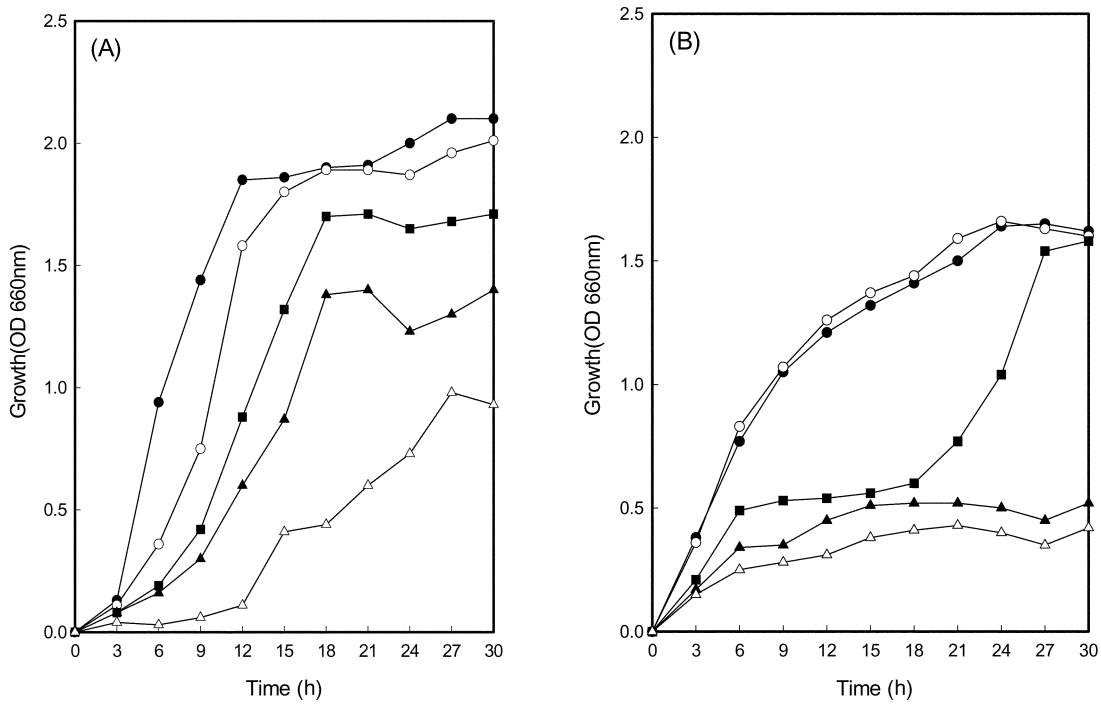

Fig. 1. Effects of the titanium-ion on the growth of *E. coli* (A) and *P. aeruginosa* (B). The strains were grown in LB medium without titanium (●), with 150 μg/ml (○), 200 μg/ml (■), 250 μg/ml (▲), and 300 μg/ml (□).

increased as cultivated time increased, and it reached its maximum cell growth after 27 h. *P. aeruginosa*'s growth gradually increased in the medium with high concentrations of titanium ion (150 μg/ml to 200 μg/ml) and also reached a maximum cell growth after 27 h (Fig. 1B). Especially, in the medium with the titanium ion concentration of 200 μg/ml, the growth of *P. aeruginosa* gradually increased for 6 h and was equally maintained at the cell population level for 12 h and thereafter, the growth suddenly increased for 9 h. IC50 values of *E. coli* and *P. aeruginosa* were calculated at 270 μg/ml and 290 μg/ml of titanium ions, respectively. An extreme cadmium-tolerant yeast, *Hansenula anomala* B-7, showed abundant growth in an aqueous medium containing 1,000 μg/ml of the cadmium ions. However, in the presence of 1,000 μg/ml of the cadmium ions, a lag phase of *H. anomala* B-7 was lengthened for 4 days, and afterwards, the data revealed a 65% population level of the cells as opposed to the case with no cadmium ions under the same conditions for 7 days (Yu *et al*., 1986). A lag phase of *Agrobacterium tumefacience* was lengthened from 3 up to 6 h in the presence of 5-10 μg/ml of the cadmium ions (Babich and Stotzky, 1977).

Cadmium (Nucifora *et al*., 1989; Yoon and Silver, 1991), arsenical (Ji and Silver, 1992; Carlin *et al*., 1995), and mercuric resistance operons (Bhriain *et al*., 1983; Yoon, 1994) are found on plasmids of bacteria, but this is not the case for the titanium resistance. Plasmid-cured *E. coli* with mitomycin C was tested to examine if

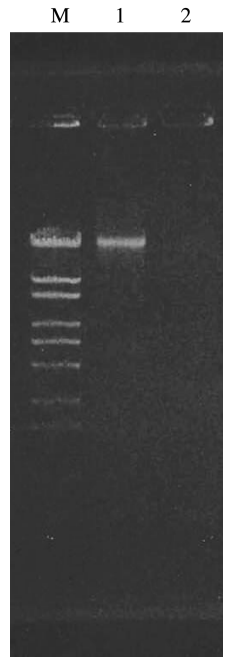

Fig. 2. Electrophoresis of plasmid DNA that was isolated from the original wild-type *E. coli*. *E. coli* K-12 (1), plasmid-cured *E. coli* K-12 (2) and marker λDNA were digested with *Eco*T14 (M).

the titanium resistance phenotype of the *E. coli* strain was caused by the acquisition of the titanium resistance plasmid. Fig. 2 shows an electrophoresis pattern of wild-type *E. coli* and plasmid-cured *E. coli*. The plasmid DNA could be isolated from the original strain but not from the

Table 2. Relative growth of wild-type *E. coli* K-12 and plasmid-cured *E. coli* K-12

Strain	Plasmid	Growth (OD_{660})	Relative growth (%)
Wild-type *E. coli* K-12	present	1.78	100
Plasmid-cured *E. coli* K-12	absent	1.75	98.3

The strains were aerobically grown at 37°C for 24 h in LB medium with 200 μg/ml of titanium ions.

cured strain. As shown in Table 2, the growth of plasmid-cured *E. coli* showed a 98.3% compared with that of the wild-type *E. coli* in the LB medium with 200 μg/ml of titanium-ions, respectively. These results suggested that titanium resistance was independent of the plasmid in *E. coli*.

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