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Conductimetric Evaluation of Antibacterial Activity of Heated Dolomite Powder

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Antibacterial activity of dolomite heated at 200-1000℃ against *Escherichia coli* was investigated using the conductance method capable of measuring the electrical conductance change caused when bacteria metabolize and produce more mobile charged molecules from large molecules. The addition of the dolomite powder heated over 700℃ delayed the detection of conductance change in the growth media. This indicates that the heated dolomite powders exhibited antibacterial activity against bacteria. The antibacterial activity were enhanced with an increase in heating temperature. Dolomite produced MgO at about 700℃, and after it produced both CaO and MgO. And, over minimal inhibitory concentration, the heated dolomite powders acted on *E. coli* in bactericidal manner.

Key words: dolomite, antibacterial activity, conductance method, magnesium oxide, calcium oxide

1. INTRODUCTION

Microbial pollution and degradation caused by microorganisms has created serious problems in various industrial fields. Inorganic antimicrobial materials have been developed as a novel technique for controlling of microbes [1]. Especially, ceramics themselves having antimicrobial activities holds considerable attention in resent year[2, 3]. However, the evaluation method for the antibacterial activity of slightly soluble or insoluble materials, such as ceramics, has not been established yet.

We have studied the antibacterial activity of metallic oxides by the conductance method. The conductance method depends on the detection of the conductance change generated by microbial growth and metabolism. The conductance change caused by bacterial growth reflects the creation of ion pairs by metabolic activity such as the conversion of glucose to lactic acid, and /or the increase in mobility caused by the cleavage of large charged molecules, such as proteins into smaller, more mobile molecules, such as amino acids[4]. Therefore, the method can provide information of bacterial growth in turbid samples, such as powder slurries, of which antibacterial activity can not be evaluated by conventional method, such as halo test or turbidometry method[4]. From the results obtained by the conductimetric assay, about ten kinds of metallic oxides that inhibit bacterial growth were found. Magnesium oxide (MgO) and calcium oxide (CaO) powder slurries acted on both gram-positive and gram-negative bacteria in a bactericidal manner[5]. Moreover, these powders were not mutagenic, but rather reduced the mutagenicity of mutagens, such as benzo[a]pyrene, 2-nitrofluorene and methylglyoxal[6, 7].

Dolomite includes $CaCO_3$ and $MgCO_3$ as the main components, and they are converted to CaO and MgO through heat treatment. Because dolomite can be orally taken, the use of the heated dolomite is not only expected to supply minerals but also to prolong the shelf life of foodstuffs. In this work, we studied the relationship between the antibacterial activity and the heating temperature using the conductance method.

2. EXPERIMENTALS

2.1 Test bacteria

Escherichia coli 745, which were stored at Tokyo Metropolitan Research Laboratory for Public Health, were used as the test bacteria. The bacteria were cultured in Brain Heart Infusion (BHI) broth (Eiken Chemicals) at 37℃ for 24 h with a reciprocal shaker. The culture was then suspended in sterile physiological saline to yield a final bacterial concentration of approximately 10^3 colony forming units (CFU)/ml.

2.2 Heat treatment of dolomite

The composition of dolomite (Kawatetsu Miming Co. Ltd.) produced in Gifu prefecture is shown in **Table 1**. The dolomite powder was heated at 200-1000℃ for 1 h in air. After milling the powders by a planetary ball mill, the average particle size of the obtained powders became approximately 5 μm. The powder was suspended with sterile physiological saline to yield a specified concentration.

Table 1 Component of dolomite.

	CaO	MgO	SiO_2	Fe_2O_3	Water
(%)	31.1	18.5	2.09	0.16	<0.5

2.3 Conductimetric assay of antibacterial activity

For measurement of the conductance change caused by bacterial growth, Bactometer® microbial monitoring system model 64 (bioMérieux VITEK) was used. This system includes a Bactometer Processing Unit (BPU) equipped with incubators, a microcomputer, a display, and a printer. A disposable module was used for the test. The module is divided into 16 individual wells, which contain paired electrodes (**Fig. 1**). The module has caps to prevent evaporation of samples during monitoring of bacterial growth.

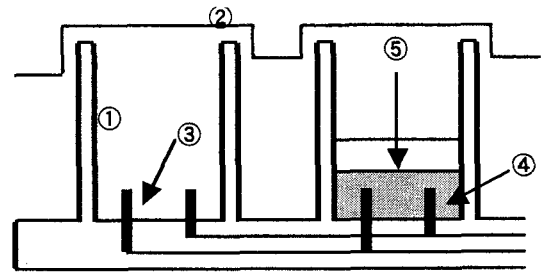
Modified Plate Count Agar (bioMérieux VITEK) was used as the growth medium for the test bacteria. Sterilized hot agar of 0.5 ml was poured into the well until it covered the electrodes. After the agar solidified, the bacterial suspension (0.1 ml) and the powder slurry (0.1 ml) were pipetted into the well. The well was closed tightly by the cap. The module was then set in the BPU, and the conductance change with bacterial growth was monitored during incubation at 35℃ for 48 h.

After monitoring, a small quantity of the slurry in the well was inoculated and incubated in BHI broth at 35℃. Whether or not the BHI broth is found to become turbid due to bacterial growth after incubation, the effect of the powder slurry was determined to be a bacteriostatic or bactericidal action, respectively.

3. RESULTS & DISCUSSION

The conductance change was detected when the test bacteria reached a threshold concentration of approximately 10^7 viable organisms per ml. The time required to reach this threshold concentration is called the "detection time (DT)". We use the DT value as a criterion for the evaluation of antibacterial activity.

Figure 2 shows the antibacterial activity of dolomite heated at 800℃ against *E. coli.* Percent conductance change of the growth medium is graphed against incubation time. The DT value of the control (powder concentration = 0 mg/ml) in Fig. 2 was approximately 6 h, meaning that it took about 6 h for *E. coli* to multiple from 10^3 to 10^7 CFU/ml. The addition of heated dolomite powder delayed the threshold of the conductance curves. The DT values at concentrations of 25 and 50 mg/ml were about 9 and 15 h, respectively. The delay of DT values indicates that the heated dolomite powder inhibited the growth of *E. coli.* An increase in powder concentration increased the DT values. The DT was not observed over 100 mg/ml. This showed that the dolomite powder heated at 800℃ completely inhibited the growth of *E. coli.*



① Well ② Cap
③ Electrode ④Agar
⑤Dolomite slurry and bacterial suspension

Fig. 1 Scheme of well

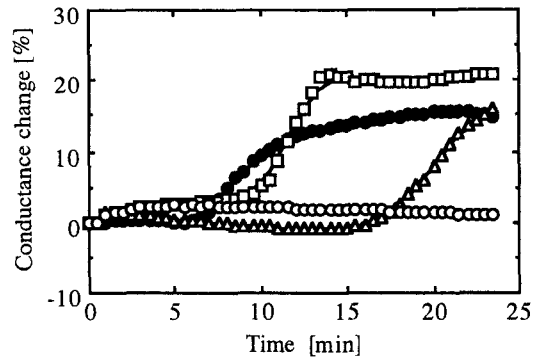


Fig.2 Effect of dolomite powder heated at 800℃ on growth of *E.coli*. ●: 0 mg/ml; □:25 mg/ml; △: 50 mg/ml; ○: 100 mg/ml.

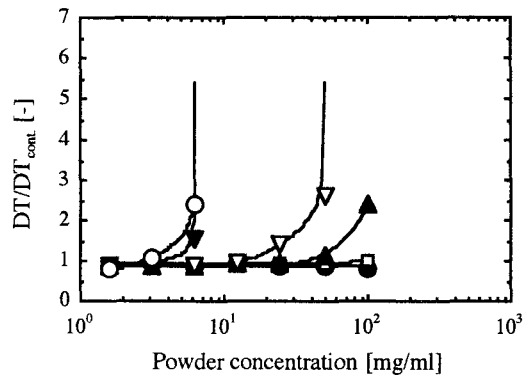


Fig.3 Effect of heating temperature on antibacterial activity of dolomite powder against *E.coli*. ●: 600 ℃; □: 650 ℃; ▲: 700 ℃; ▽: 800 ℃; ▼: 900 ℃; ○: 1000 ℃.

The effect of heating temperature on the antibacterial activity of dolomite powder is shown in **Fig. 3**. The abscissa $DT/DT_{cont.}$ represents the ratio of the DT at the

Table 2 Minimal inhibitory concentration of heated dolomite powder against *E. coli*.

Minimal inhibitory concentration [mg/ml]					
600℃	650℃	700℃	800℃	900℃	1000℃
-	>100	>100	50	12.5	12.5

-: No inhibition

specified powder concentrations to that at 0 mg/ml (control). The powder whose curve showed a steep rise at a lower concentration exhibits more antibacterial activity against *E. coli*. When the powder does not show the antibacterial activity, the value of $DT/DT_{cont.}$ is unity. The $DT/DT_{cont.}$ values of the powders did not change up to 650℃, indicating that the powders heated at lower than 650℃ exhibited no antibacterial activity. And, the increases in the DT values were observed over 700℃. Generally, under atmospheric pressure, thermal decomposition of the dolomite produces MgO and CaO at approximately 700℃ and 900℃, respectively[8]. The first change in DT values for the powder heated at 700℃ was corresponding to the generation of MgO. At over 800℃, it was considered that the coexistence of MgO and CaO enhanced the antibacterial activity. Okouchi *et al.* [9, 10] also reported the two step occurrences of antibacterial activity of heated dolomite using the calorimetry. Therefore, by varying the sintering temperature, there is a possibility of controlling the antibacterial activity.

Table 2 shows the variation of minimal inhibitory concentration (MIC) of the dolomite powders heated at different temperatures. MIC presents the minimum concentration of powder slurry where the DT is not detectable with the measurement of conductance. The MIC decreased with an increase in heating temperature. From the results of incubation with BHI broth, the dolomite powders exhibited a bactericidal action against *E. coli* over MIC.

The antibacterial factors of heated dolomite powder have not been cleared yet. However, there are a few works concerning with alkaline earth metallic oxides (MgO and CaO), which are the main components of the heated dolomite. Both MgO and CaO powder slurries have high pH value, about 10 and 12, respectively. However, the bactericidal actions of MgO and CaO powder slurries were larger than those of NaOH solution with the same pH as the slurry[11]. In addition, the changes in the bacterial antibiotic sensitivities by the CaO and MgO powder slurries were obviously different from those by alkaline treatment [11]. For the MgO and CaO, the generation of active oxygen, such as superoxide anion, was observed from the powder slurries by chemiluminescence analysis[12]. The order of the strength of luminescence response was CaO and MgO, which agreed with that of the antibacterial activity. The antibiotic sensitivity changes by these powder slurries almost agreed with those by active oxygen treatment[13, 14]. Although the alkaline effect will be the primary factor, these active oxygen species may also act on bacteria as one of the bactericidal factors apart from the alkaline. A rise of heating temperature produces MgO and CaO, and makes dolomite antibacterial. This will be due to the increase in the alkalinity of dolomite and in the generation of active oxygen with an increase in heating temperature.

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