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Influence of Food Components on MgO-Induced Bacterial Death

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The influence of various additives on the bactericidal action of MgO powder slurry against *Escherichia coli* was investigated to obtain information about magnesium oxide (MgO)-induced bacterial death in the presence of food components. Addition of K^+, Na^+, and Ca^{2+} to the MgO powder slurry promoted the death of *E. coli*. Ca^{2+} was particularly effective at low concentrations. Although Mg^{2+} is also a divalent cation, it did not affect the bactericidal action of MgO. In the case of saccharides, both glucose and maltose reduced the bactericidal action of MgO, but soluble starch had no effect. Substances related to protein and fatty acids hindered the bactericidal action.

Key words: antibacterial activity, magnesium oxide, metallic ion, saccharide, protein

1. INTRODUCTION

Several attempts are currently underway to employ inorganic antimicrobial materials for prevention of bacterial contamination and biodegradation that occur in many fields[1-4]. Most of these methods use ceramic materials, such as zeolite and hydroxyapatite, as supports, and antimicrobial agents, such as silver and copper compounds, are immobilized on the surface of the support [5-8]. The antimicrobial activity of these materials originates from the antimicrobial agents present on the surface, and is not due to the ceramic itself. Although these materials are effective, there are some problems associated with their use, such as the appearance of silver-resistant bacteria and allergies[9]. Recently, the use of a ceramic that itself displays antimicrobial activity has attracted much attention[10-12].

We evaluated the antibacterial activity of 26 ceramic powders by the conductance method which detects bacterial growth as a change in electrical conductance, and about ten powders were found to inhibit bacterial growth. In particular, magnesium oxide (MgO), calcium oxide (CaO) and zinc oxide (ZnO) exhibited strong antibacterial activity. MgO and CaO powders were effective against both gram-positive and gram-negative bacteria in a bactericidal manner. ZnO powder inhibited the growth of gram-positive bacteria more strongly than gram-negative bacteria[13]. In addition, these powders have also been seen to be effective against the spores of *Bacillus subtilis*, which are highly resistant to heat and antimicrobial agents[14]. Furthermore, these powders were not mutagenic, but rather reduced the mutagenicity of mutagens such as benzo[a]pyrene, 2-nitrofluorene and methylglyoxal[15,16].

These metallic oxides can be taken orally. The use of these materials is therefore not only expected to prolong the shelf life of foodstuffs but also to be a source of minerals. However, there have been few quantitative studies on the antibacterial activity of these metallic oxides[17]. Before these metallic oxides are actually used in food processing, it is necessary to study the effect of food components on antibacterial activity. In the present study, the influence of various additives on the bactericidal action of MgO against *Escherichia coli* is examined.

2. EXPERIMENTAL

2.1 Test organism

Escherichia coli 745 obtained from the Tokyo Metropolitan Laboratory of Public Health was used throughout this work. The bacteria were stored at -80℃, then thawed and cultured in Brain Heart Infusion broth (Eiken Chemicals) at 37℃ for 20 h. The culture was cooled in ice water before being used for MgO powder slurry treatment.

Table 1 The additives to MgO powder slurry

Metallic ions (chlorides)	NaCl, KCl, $MgCl_2$, $CaCl_2$
Saccharides	glucose, maltose, starch
Substances related to protein	peptone, yeast extract
Fatty acids	oleic acid, olive oil, corn oil

2.2 MgO powder slurry treatment

MgO (Kishida Chemicals) powder with a mean particle size of 3.6 μm was heated to 180℃ for 20 min and suspended with sterile distilled water to yield a powder slurry at a concentration of 5.0 mg/ml. As shown in Fig. 1, the powder slurry (300 ml) was poured into a reactor with an inner diameter of 85 mm, and agitated with a magnetic stirrer at 250 rpm. The additives shown in Table 1 were added to the slurry. The slurry temperature was controlled at 37℃ using a water bath. The bacterial culture (0.3 ml) was pipetted into the slurry and treatment was started.

2.3 Viable counts

The sample (0.1 ml) was periodically removed, diluted with saline and cooled in ice water. The diluted samples were pour-plated with Nutrient Agar (Eiken Chemicals). Duplicate plates were used for each dilution. The colonies were counted after incubation at 37℃ for 48 h.

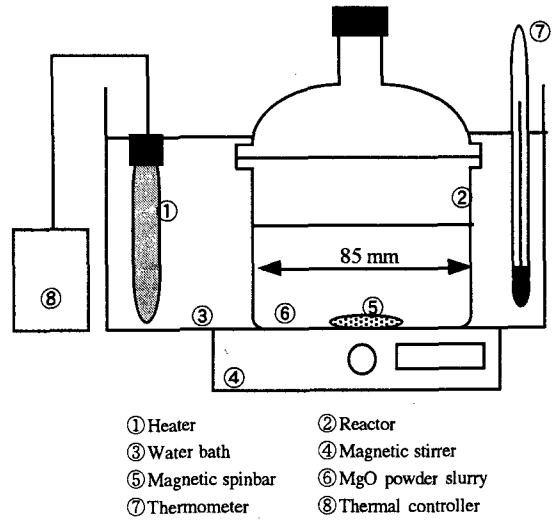

Fig. 1 Schematic drawing of experimental apparatus.

3. RESULTS & DISCUSSION

3.1 Effect of metallic ions

Figure 2 shows the effect of Na^+ on the bactericidal action of MgO powder against *E. coli*. Addition of Na^+ significantly promoted bacterial death, and the bactericidal action of MgO was seen to increase with an increase in Na^+ concentration. Only NaCl in the concentration range employed here did not produce a

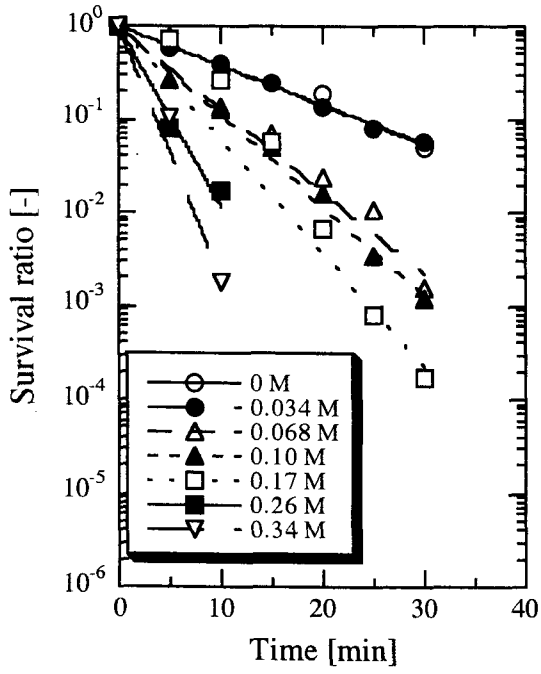

Fig. 2 Effect of Na^+ (NaCl) on bactericidal action of MgO powder slurry.

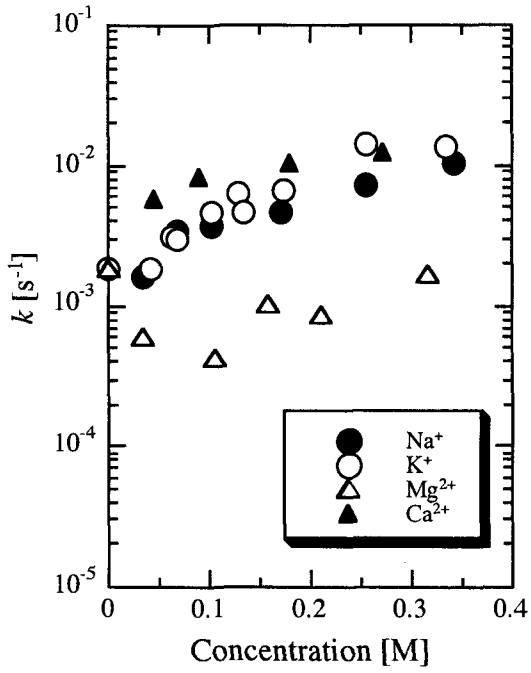

Fig. 3 Effect of metallic ions on bactericidal action of MgO powder slurry.

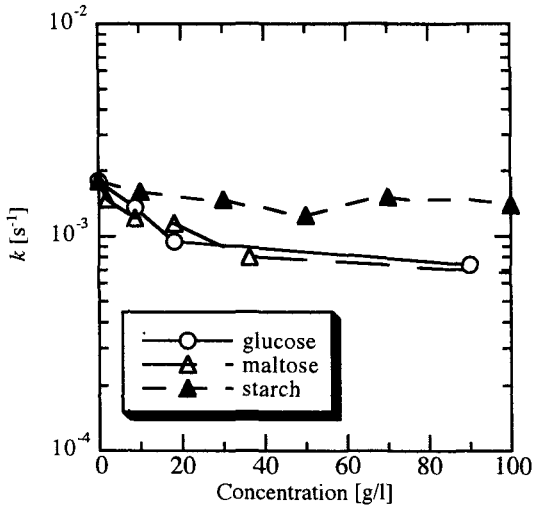

Fig.4 Effect of saccharides on bactericidal action of MgO powder slurry

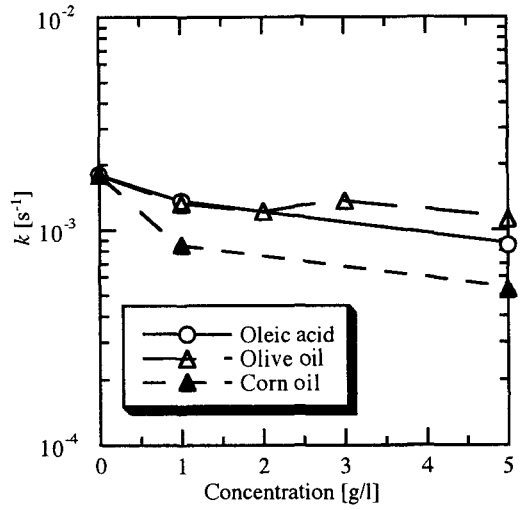

Fig.5 Effect of fatty acids on bactericidal action of MgO powder slurry

decrease in the survival ratio of *E. coli*. As shown in Fig. 2, *E. coli* death follows a first-order reaction expressed by the following equation.

$$dN/dt = -kN \quad (1)$$

where N is the concentration of viable *E. coli* cells, and k is the apparent first-order death rate constant.

To compare the effects of metallic ions, k value variations caused by the addition of metallic ions were examined (Fig. 3). Na^+, K^+ and Ca^{2+} increased the bactericidal action of MgO powder slurry. In particular, Ca^{2+} was highly effective at low concentrations. Although Mg^{2+} is also a divalent cation, its addition did not affect the bactericidal action of MgO powder slurry. This can be probably attributed to the fact that the presence of Mg^{2+} lowered the solubility of MgO ($Mg(OH)_2$) in water.

3.2 Effect of saccharide

Figure 4 shows the effects of saccharides on the bactericidal action of MgO powder slurry. Glucose and maltose reduced the k values of MgO. However, no effect was observed in the case of starch. It is thought that the decrease in water activity caused by the addition of glucose or maltose is greater than that due to the addition of strach[18].

3.3 Effect of fatty acids

Figure 5 shows the effects of fatty acids on k values of MgO. Highly unsaturated fatty acids, such as linolenic acid in corn oil, prevented a decrease in the survival ratio of *E. coli* more strongly than oleic acid and olive oil.

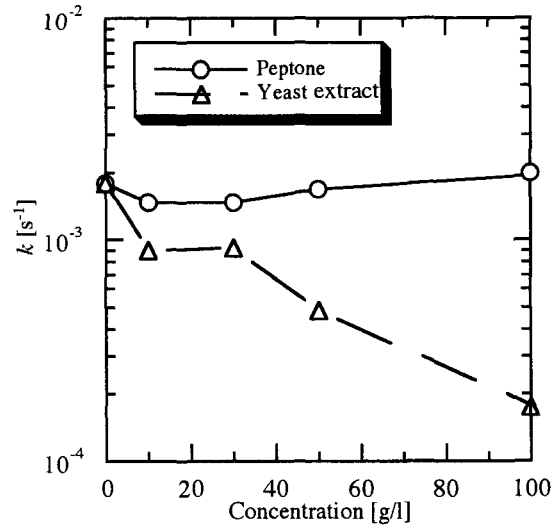

Fig.6 Effect of substances related to protein on bactericidal action of MgO powder slurry

3.4 Effect of substances related to protein

Peptone and yeast extract were used as substances related to protein. A decrease in bactericidal action was observed for peptone but not yeast extract (Fig. 6). This may be due to choline, which is a compatible solute and the precursor of glycinebetaine in yeast extract[19].

At the present stage, the action mechanism of metallic oxide powders is not yet fully understood. However, the production of active oxygen, such as superoxide (O_2^-), has been previously observed from MgO powder slurry [20], and it has been suggested that active oxygen generated from MgO powder slurry is one of the primary factors influencing antibacterial activity[21].

Acknowledgements

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