Various Plating Metals & Their Antimicrobial Effect

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The food poisoning by pathogenic microbes has caused serious problems, particularly large-scale outbreak of enterohaemorrhagic *Escherichia coli* O157:H7 infection in Japan, 1996. Therefore, many antisepsis procedures and prevention ones have been proposed as a countermeasure to improve facilities for providing meals. Since we have very few data base for the interactions between microbes and metals, there are still few trials to give the materials surfaces an antimicrobial effect only by metal plating itself. Then we observed that growth of some microbes was inhibited by plating metals. From these results, we discussed on the mechanism of antimicrobial effect by metals.

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INTRODUCTION

It is already well known that some metals such as silver, copper, zinc, cobalt etc. have a sort of antibacterial actions. And many researchers and engineers have not only studied on them academically, but also developed various products utilizing antibacterial metals[(1)-(3)]. When our purposes are focused on the antibacterial actions of metals, it is adequate for us to give some antibiotic actions on the materials surfaces. However, there are few studies and researches from the viewpoints of metallic surfaces. Therefore, we focused on the metallic surfaces in terms of antibacterial actions and investigated the antimicrobial activities of some plating metals. Then we produced plating films on steels for some confirmed antimicrobial metals and checked the antimicrobial activities for the plated steels.

EXPERIMENTAL

Bacteria and agar medium

Escherichia coli ATCC25932, *Staphylococcus aureus* 209P and *Klebsiella pneumoniae* ST101 were used as bacteria in this experiment. The cultures for the inocula were incubated in nutrient broth (10mL) in test tubes on a rotary shaker (150 rpm) for 6h at 37 degrees Celsius (98.6 degrees Fahrenheit). The concentration of bacterial suspension was adjusted to 10^6/ml with physiological saline, based on optical density of the culture.

Ordinary agar medium was used for their incubations. Metal powders (aluminum, chromium, magnesium, titanium, manganese, cobalt, nickel and copper) in a certain weight were put into test tubes and mixed with ordinary agar medium (10mL). After sterilization by stem at high temperature and high pressure at 121 degrees Celsius (249.8 degrees Fahrenheit) in 20 minutes, they were mixed by a mixer enough and used as agar medium including metal powders. 18 hours after the inoculation, the numbers of colony formation unit (CFU) were measured by naked eyes. Generally speaking, the number of CFU is equal to that of original bacteria. Therefore, the former was used as vertical axis. However, they were the number of CFU, in fact.

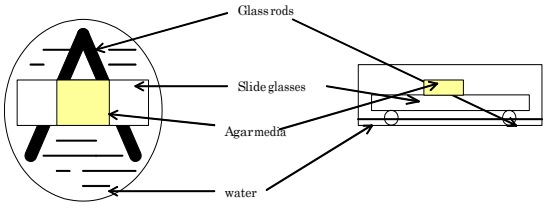

Fig.1 Schematic illustration for slide culture technique

The slide culture method was used to investigate the interaction between plated metals and bacteria. It was shown schematically in Fig.1. A V-shape glass rod was put in a petri dish and a slide glass was put on it so that the slide glass did not touch the bottom of the petri dish. The petri dish was sterilized by hot air in an hour. Then a medium where one of the bacteria was inoculated was put on the slide glass and a plated steel was put on the top of them. The bottom of the petri dish was filled with small quantity of water to protect the dehydration during culture. The petri dishes were kept at 37 degrees Celsius (98.6 degrees Fahrenheit) for 24 hours in an incubator, and the breeding of bacteria was observed.

Plating and Substrates

Steels (JIS SS400) were cut into small plates (10 H 10 mm: 3.94 H 3.14 in) and degreased by immersing in methanol (75%) for 30 minutes. Then a lead wire was attached to a side of the each specimen electrically. Except for a side of the specimen as electrode surface, all of other sides were coated by insulative resin.

Table 1 The composition of the solution for zinc plating

Zinc sulfate	$ZnSO_4$	150g
Boric acid	H_3BO_3	9g
Aluminum sulfate	$Al_2(SO_4)_3$	3g
Sodium chloride	NaCl	9g
Purified water		300ml

To investigate the antibacterial properties for plated steels, we plated steels with zinc and copper. Table 1 and 2 show the compositions of solutions for zinc and manganese plating, respectively. In both cases, specimens were used as cathode and insoluble electrodes as anode. The initial current and voltage for plating were 0.1A and 3.7V and plating time was 5 minutes for all cases. On the other hand, commercial items were used for copper plating.

RESULTS AND DISCUSSION

Antimicrobial Activities of Various Metal Powders

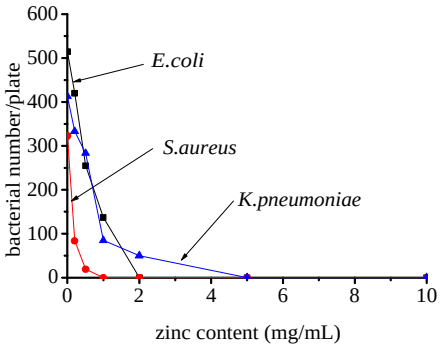

Fig.2 Bacterial growth with zinc powder in agar media.

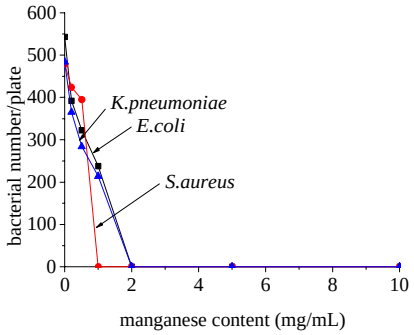

Fig.3 Bacterial growth with manganese powder in agar media.

Firstly, we investigated how these three bacteria (*E.coli*, *S.aureus*, *K.pneumoniae*) grew on ordinary agar medium mixed with metal powders such as zinc, manganese, nickel, copper, cobalt, chromium, titanium, aluminum, silver, magnesium and iron.

Fig.2 shows the change of bacteria numbers with the addition of zinc powder in the ordinary agar medium. For all these three bacteria, the numbers decreased with the zinc content drastically. For *E.coli*, the number of bacteria reached zero, when the zinc content became 1mg/mL. For *K.pneumoniae* whose antibiotic action by zinc was the lowest, the number reached zero, when the zinc content became 5mg/mL.

Fig.3 shows the change of bacteria numbers with the addition of manganese powder in the ordinary agar medium. In this case, the antibacterial effect of the metal was higher than that in Fig.2. All of these bacteria became extinct drastically when the content of manganese powder reached 2 mg/mL. *S.Aureus* showed the most remarkable antibacterial effect for manganese powder.

Fig.4 shows how the bacteria numbers changed with the addition of nickel powder in the ordinary agar medium. For all of these three bacteria, the numbers decreased drastically when the content of nickel increased in the ordinary agar medium. All of these three bacteria became extinct until the content reached up to 2mg/mL.

Fig.5 shows the decrease of bacteria numbers with the increased content of copper in the ordinary agar medium. The metal powder also showed an antibacterial effect on all of these three bacteria. Any of these bacteria decreased drastically, when the content of copper in the medium increased. However, the copper content in the medium to make *K.pneumoniae* distinct was

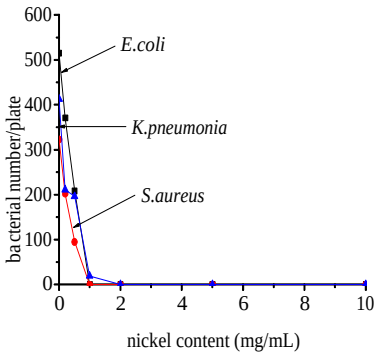

Fig.4 Bacterial growth with nickel in agar media.

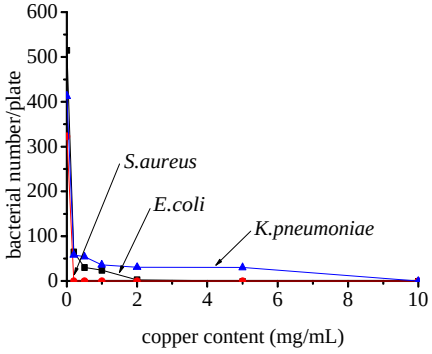

Fig.5 Bacterial growth with copper in agar media

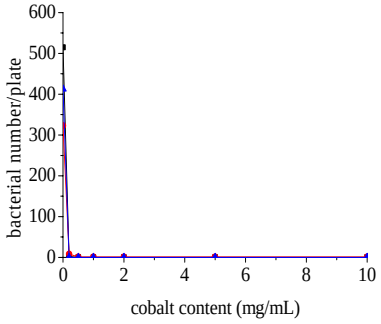

Fig.6 Bacterial growth with cobalt in agar media

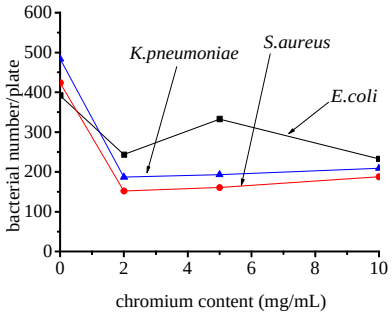

Fig.7 Bacterial growth with chromium in agar media

relatively higher (10mg/mL).

Fig.6 shows the change of bacteria numbers with the addition of cobalt in the medium. In this case, the addition into the medium shows a remarkable antimicrobial effect on all of these bacteria. For all cases, the bacterium numbers decreased drastically when the cobalt content increased.

Fig.7 shows how the bacteria numbers changed with the content of chromium in the agar medium. As shown in the figure, chromium has the antimicrobial activity to some extent, since the bacterial numbers decreased with the addition of chromium powder. However, it did not make any of these bacteria extinct completely. It suggests that chromium does not have so remarkable effect for antimicrobial activities against these bacteria. .

Fig.8 shows the correlation between the bacteria numbers and the content of titanium in the agar medium. Even though the numbers of all bacteria decreased with the addition of titanium in the ordinary agar, they did not become distinct. Therefore, we can conclude that it shows the antimicrobial activity to some extent, however, it did not show the significant antimicrobial activity for these three bacteria.

Fig.9 shows the change of bacteria numbers with the content of aluminum powder in the ordinary agar. The metal also decreased the numbers of these bacteria. From the viewpoint, it has the antimicrobial capability to some extent. However, it did not make them distinct completely. Therefore, we also judged that aluminum powder shows a weak antimicrobial activity for any of these bacteria.

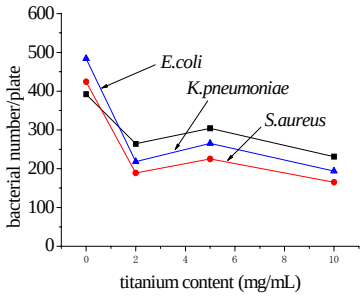

Fig.8 Bacterial growth with titanium in agar media

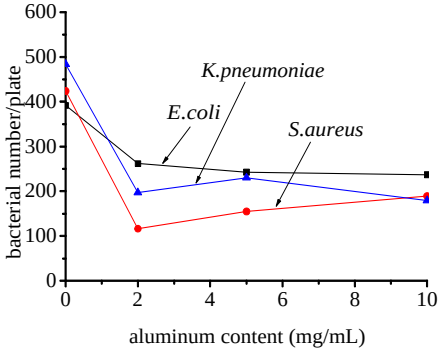

Fig.9 Bacterial growth with aluminum in agar media

Fig.10 shows how the silver powder affected the bacterial numbers with the increased contents. For all bacteria, the addition of silver decreased the numbers. *S.Aureus* was the most sensitive to the antimicrobial activity of silver and the microbial activity was the lowest for *K.pnemoniae*. Generally speaking, silver has been well known for it=s strong antimicrobial activity. However, it did not show so strong antimicrobial activity for these three bacteria, even though it has the antimicrobial activity to some extent.

Fig.11 shows the correlation between bacterial numbers and the content of magnesium powder in agar medium. As shown in this figure, any of these bacterial numbers did not decrease with the increased content of magnesium at all, even though they fluctuated within a content range. We can conclude that magnesium powder did not show any antimicrobial activity at all.

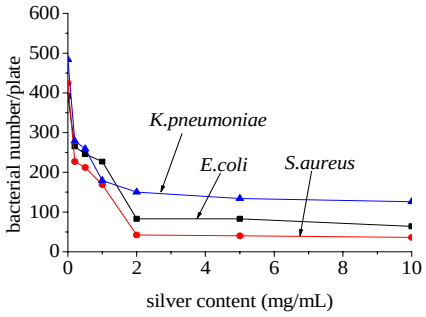

Fig.10 Bacterial growth with silver in agar media

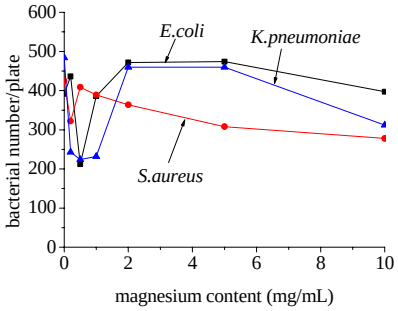

Fig.11 Bacterial growth with magnesium in agar media

Fig.12 shows the correlation between the bacterial numbers and the content of iron powder. Even though iron showed the antimicrobial activity for *K.pneumoniae* to some extent, the other two bacteria did not show any antimicrobial activities by iron powder at all. Since iron is often needed for bacteria to maintain their lives, it is very natural for iron not to show any antimicrobial activity.

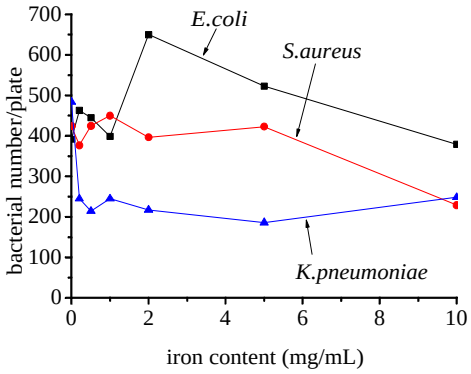

Fig.12 Bacterial growth with iron in agar media

All of these results indicate that zinc, manganese, nickel and copper have the stronger antimicrobial activity for these three bacteria, and that chromium, titanium, aluminum and silver have the weak antimicrobial activity. They show that the antimicrobial activity of iron and manganese are very low for these three bacteria.

Antimicrobial Activities of Zinc Plated Steels

We observed the growth of bacteria at 37 degrees Celsius (98.6 degrees Fahrenheit) in 18 hours by slide culture technique and investigated how the zinc plated steels affected the growth of bacteria.

	Escherichia coli ATCC25932	*Staphylococcus aureus* 209P	*Klebsiella pneumoniae* ST101
Without zinc plated specimens	 ①	 ②	 ③
With zinc plated specimens	 ④	 ⑤	 ⑥

Fig.13 Growth conditions of bacteria with zinc plated specimens.

Fig.13 shows what the growth conditions of these bacteria looked like. Fig.13-(1), (2) and (3) correspond to the surface conditions of agar media on the slide glasses to which we applied each bacterium. In any cases, bacteria grew enough on the whole surfaces.

On the other hand, Fig.13-(4), (5) and (6) correspond to the surfaces of agar media, when zinc plated steels were put on the media. For the agar medium with *E.coli* (Fig.13-(4)), the bacterium free zone was found around the zinc plated specimen. For those with *S.aureus* (Fig.13-(5)) and *K.pneumoniae* (Fig.13-(6)), the growth did not almost occur on the entire of the surfaces. These results indicate that zinc plating could inhibit the growth of these three bacteria. When we focus on the extent of the inhibition effect for all of these three bacteria, we can realize the difference of the inhibition extent among these bacteria. The inhibition extent for *S.aureus and K.pneumoniae* was much higher than that for *E.coli*. Red circles in Fig.15-(3), (4) and (5) show the areas where white precipitations were observed. They were zinc hydroxides formed through the plated specimens and agar media.

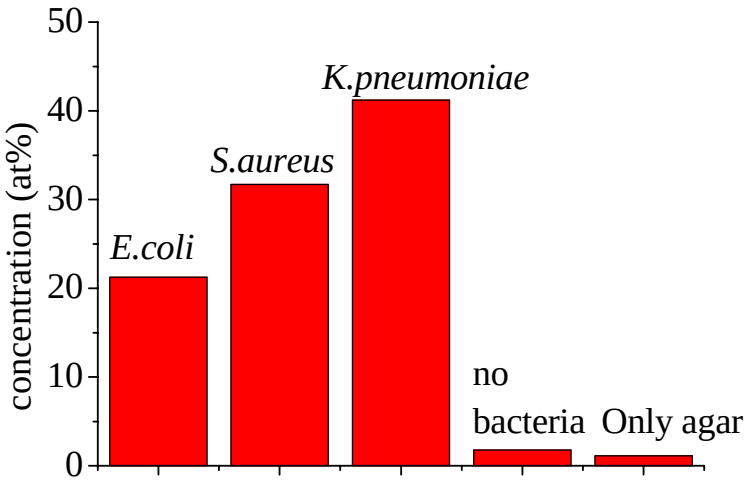

Fig.14 zinc contents in agar media analyzed by fluorescent X-ray analysis.

We presume that the inhibition effect by zinc plating on the bacteria growth was caused by the zinc ion dissolved into agar media. We analyzed the agar media by fluorescent X-ray analysis (FXRA) to confirm the assumption.

Fig.14 shows the zinc contents in agar media analyzed by FXRA, when the zinc plated steel was put on the agar medium inoculated by each of these three bacteria. The result for the agar media with the zinc plated specimen, but without bacteria was shown as reference in the figure. And another result for the agar medium without zinc plated specimen and bacteria was also shown in the figure as reference. The agar media were analyzed by fluorescent X-ray after they were cultured at 37

	Escherichia coli ATCC25932	*Staphylococcus aureus* 209P	*Klebsiella pneumoniae* ST101
Without copper plated specimens	 ①	 ②	 ③
With copper plated specimens	 ⑦	 ⑧	 ⑨

Fig.15 Growth conditions of bacteria with copper plated specimens.

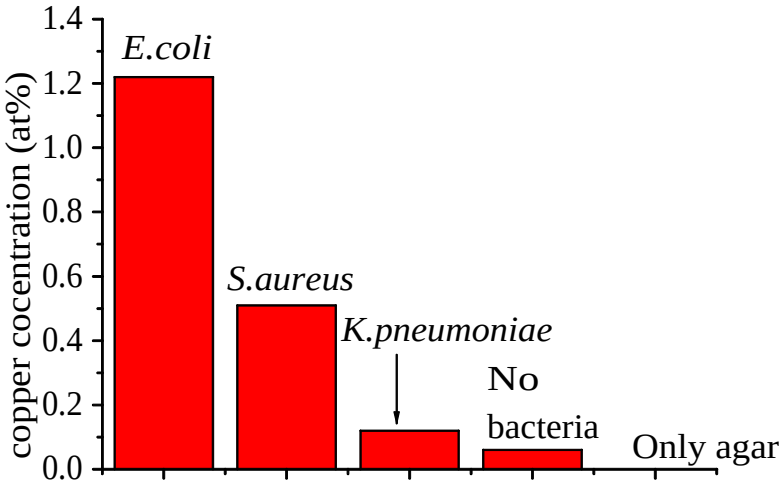

Fig.16 Copper contents in agar media analyzed by fluorescent X-ray analysis.

degrees Celsius ((98.6 degrees Fahrenheit) in 18 hours. The results indicate that the agar media with zinc plated specimens and bacteria contained zinc 30 or 40 times more than the agar with bacteria, even though the latter also included a same zinc plated specimen. The amount of zinc dissolved in the agar medium decreased in this order: *K.pneumonia*, *S.aureus* and *E.coli*. The order for dissolved amount of zinc corresponds to that of the inhibition effect for the bacteria growth. It suggests that zinc played an important roll to the antimicrobial activity of bacterial growth.

Fig.15 shows the growth conditions of bacteria in agar media, when copper plated specimens co-existed with bacteria in the agar media. When the bacteria were inoculated in the media without copper plated specimens, any of these bacteria grew enough on the whole areas as shown in Fig.15-(1), (2) and (3). On the other hand, when the copper plated specimen was put on the agar including bacteria, the growth conditions were changed shown in Fig.15-(4), (5) and (6). For *S.aureus* (Fig.15-(5)), a bacteria free zone formed around the copper plated specimen and it affected the growth of bacteria also on the entire surface. For both *E.coli and K.pneumoniae* (Fig.15-(4) and (6)), bacteria free zones also formed around the copper plated specimens, while the bacteria could grow enough in the areas away from the plated specimens. These results suggest that the copper plated steel has the inhibition effect for the bacteria growth and also that the extent of the antimicrobial activity is higher for *S.aureus* than that for both *E.coli* and *K.pneumoniae.*

Fig.16 shows the results of fluorescent X-ray analysis for agar media. It indicates that copper dissolved into the agar, when the copper plated specimens were placed in the agar media. The extent of dissolution differed from the sample to sample. The amounts of copper in the agar media with bacteria were more than that without bacteria. The amount of copper in the media decreased in this order: *S.aureus*, *E.coli and K.pneumoniae*, which corresponds to the high antimicrobial

activity of copper for *S.aureus*. The results for copper specimens being compared with those for zinc plated specimens, we can conclude that the absolute figures for the amount of dissolved copper in the agar media were much smaller than those for dissolved zinc. It indicates that copper can show antimicrobial activity at much smaller quantities than zinc.

As mentioned above, the amounts of dissolved zinc and copper were much more under the co-existence of bacteria than those in the agar media without bacteria. We presume that the metabolites by bacteria promoted the dissolution of these metals into agar media. Once the metal ions such as copper ion and metal ion dissolve into agar media including bacteria, they begin to decrease the activities of proteins and may produce cytotoxic active oxygen. Therefore, the metal powders dissolved into the agar media by the metabolism of bacteria cells and the ionized metals caused the antimicrobial activities.

In this paper, only zinc plating and copper plating were investigated for their antimicrobial activities. However, other metals which could show antimicrobial activities can show the same tendencies, when they are plated on materials. In such a case, it will be an important key how much the metal can dissolve into media as ion.

CONCLUSIONS

We carried out a series of experiments where we added some metal powders into agar media with *E.coli, S.aureus and K.pneumoniae,* respectively and investigated what kind of metals could show antimicrobial activities against these three bacteria. From the results, we elucidated that zinc, manganese, nickel and copper have the strong antimicrobial activity against these bacteria. And we also figured out that chromium, titanium, aluminum and silver have the weak antimicrobial activity, while magnesium and iron don=t show any significant antimicrobial capability. Then we chose zinc and copper among these antimicrobial metals and produced their plated steels by electroplating. We observed the inhibition effects of these plated specimens against the growth of these bacteria by slide culture technique. As a result, we figured out that both plated specimens had antimicrobial activities for these three bacteria. The metabolism of bacteria induced the dissolution of metals into the media as ion and metallic ions brought bacteria cytotoxic effects by decreasing the activity of proteins.

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