Antibacterial Activity by Alloying of Tin & Copper Plating

Hajime lkigai & Hideyuki Kanematsu, Suzuka National College of Technology, Suzuka, Japan;
Kensuke Kuroda; Nagoya University, Nagoya, Japan
& Akira Ohmori, Osaka University

The antibacterial activities of some plated metals have serious implications for applications such as medical instruments, food processing, etc. We have already found that zinc and copper electroplates exhibit strong antimicrobial activity versus *E-coli, Staphylococcus aureus, Klebsiella pneumoniae, etc*. On the other hand, tin plating has commonly been applied to food processing applications. Therefore, using slide culture techniques and film adhesion tests, we studied the antibacterial activity of tin plating and observed how the characteristics change with the extent of alloying between tin and copper.

For more information, contact:
Hajime Ikigai, Ph.D, MIMF
Professor, Dept. MS & E.
Suzuka National College of Technology:
Shiroko-cho, Suzuka, Mie 510-0294, Japan
Voice/FAX: +81-593-68-1849
email: kanemats@mse.suzuka-ct.ac.jp
Web: http://www1.mint.or.jp/~reihidek

INTRODUCTION

Some metals such as silver, copper etc. are well known for their antibacterial action. The information leads not only to the academic investigation, but also to the practical application to "antibacterial goods" We have investigated the antimicrobial characteristics of various metal powders against some bacteria such as *Escherichia Coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* and found that some important plating metals such as copper, zinc etc. have high antibacterial actions[(1),(2)]. However, the antibacterial properties for tin which has been applied to food processing very often have not been clarified yet. Therefore, we investigated the antibacterial action of the tin and copper plating against these three bacteria using slide culture technique and film adhesion technique in this study. And then we investigated also the antibacterial properties of the alloy plating between these two elements.

EXPERIMENTAL

Bacteria used in this study

Bacterial used in this investigation were *Escherichia coli* ATCC25932 (*E.coli*), *Staphylococcus aureus* 209P (*S.aureus*) and *Klebsiella pneumoniae* ST101 (*K.pneumoniae*).

Antibacterial tests

The evaluations for antibacterial properties were carried out in the two ways: Slide culture technique and film adhesion one.

For slide culture technique, 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 plated steels were 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 dish was kept at 37 degrees Celsius (98.6 degrees Fahrenheit) for 24 hours in an incubator, and the breeding of bacteria was observed. The schematic figure for this technique was shown in Fig.1.

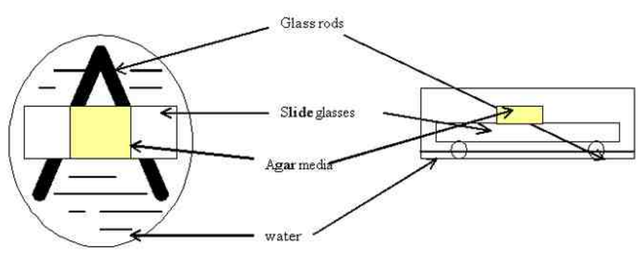

Fig.1 Schematic illustration for slide culture technique

For film adhesion tests, these bacteria were pre-incubated in 10ml of nutrient broth for 18h at 35 degrees Celsius (95 degrees Fahrenheit) and then diluted to 10^5/ml with 0.9% NaCl solution. Four hundred micro litters of each bacterial suspension were put on metal plates (5 x 5cm: 2 x 2 inch). A sterile polyethylene film (4 x 4cm: 1.6 x 1.6 inch) was immediately covered on the bacterial solution. This metal plate transferred to a sterile plastic petri dish was kept for 24h at 35 degrees Celsius (95 degrees Fahrenheit) under relative humidity above 99%. Bacteria adhered to a film and a metal plate, respectively, were washed out with pipetting after addition of 10ml of 0.9% NaCl containing 0.2% Tween 80 in the plastic petri dish. A 100 μL of the washed-out bacterial suspension was inoculated on a nutrient agar plate and spread out by a glass spreader. Then the plate was incubated overnight at 35 degrees Celsius (95 degrees Fahrenheit) and bacterial colonies were counted.

RESULTS AND DISCUSSION

Slide Culture Tests for Copper Plating for copper plated specimens

(1) *E.coli* (2) *S.aureus* (3) *K.pneumoniae*

Fig.2 Cultures' condition without specimens.

Fig.2 shows the result for slide culture tests for the control. In this case, any plated specimens were not put on the cultures. 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.2-(1), (2) and (3). On the other

(1) *E.coli* (2) *S.aureus* (3) *K.pneumoniae*

Fig.3 Growth conditions of bacteria with copper plated specimens

hand, when the copper plated specimen was put on the agar including bacteria, the growth conditions were changed shown in Fig.3-(1), (2) and (3). For *S.aureus* (Fig.3-(2), a bacteria free zone also formed around the copper plated specimens and it affected the growth of bacteria also on the entire surface. For both *E.coli* and *K.pneumoniae* (Fig.3-(1) and (3)), 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 the extent of the antimicrobial activity is higher for *S.aureus* than that for both *E.coli* and *K.pneumoniae*.

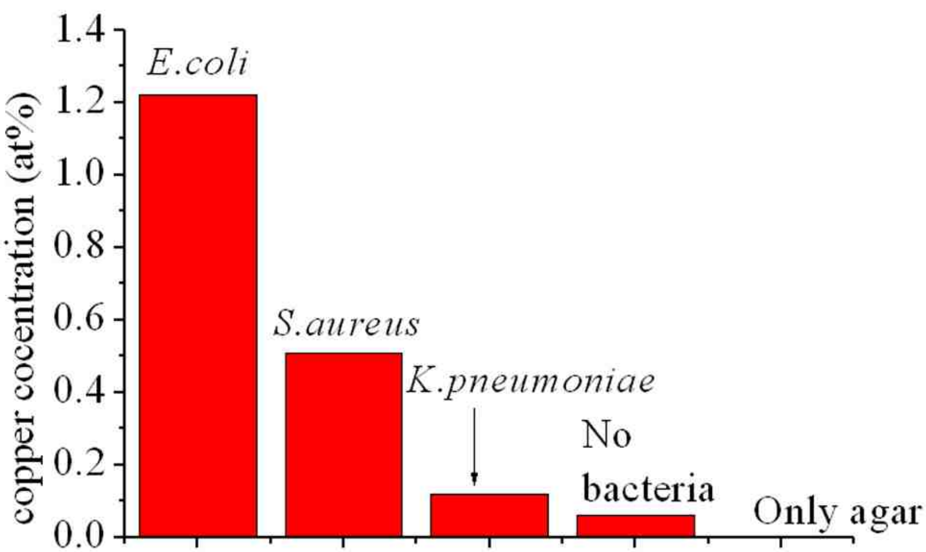

Fig.4 Copper contents in agar media analyzed by fluorescent X-ray analysis.

Fig.4 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*.

Fig.5[4] shows the schematic illustration to explain how the metal behaves as antimicrobial factor against some 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 dissolve into agar media including bacteria, they

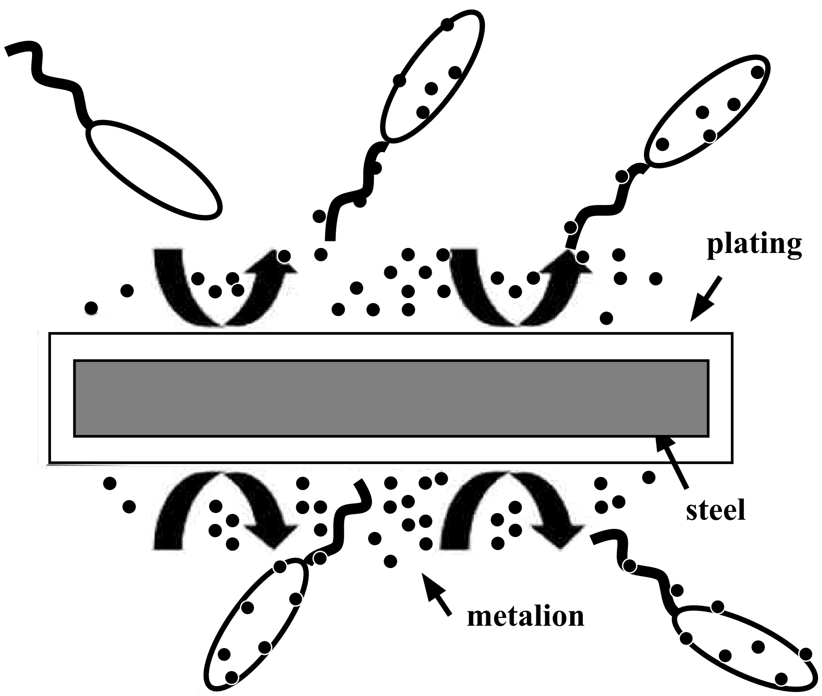

Fig.5 Mechanism of antibacterial activity by plating.

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 bacterial cells and the ionized metals caused the antimicrobial activities.

Film Adhesion Tests for Tin-Copper Alloy Plating

According to the slide culture tests, the copper plated specimen has very high antimicrobial characteristics against these three bacteria. Generally speaking, tin plating is used much more often for food processing applications. Therefore, we investigated the antimicrobial behavior of tin plating. As the assessment technique, film adhesion tests are generally more universal. Therefore, we selected this technique to evaluate the antimicrobial activities.

Firstly, we confirmed the antimicrobial characteristics of copper plating once again by this film adhesion test. Table 1 shows the results for it.

Table 1 Film Adhesion Tests For copper plating specimens.

Time (h)	Samples	*Escherichia coli* ATCC25032	*Klebsiella pneumoniae* ST101	Staphylococcus aureus 209P
0	Control	1.00×10^5 / a plate	1.83×10^5 / a plate	2.59×10^5 / a plate
24	Control	1.02×10^6 / a plate	1.43×10^6 / a plate	3.19×10^4 / a plate
	Metal plate	0	0	0

Table 1 indicates that the copper plating specimen showed the very strong antimicrobial characteristics against all of these bacteria and the result coincided with that by the slide culture technique completely.

Then we investigated the antimicrobial characteristics of the specimen which have the tin top layer with the copper under layer on the steel substrate by this film adhesion test. Table show the results for this kind of specimens.

Table 2 Film Adhesion Tests for Tin (top layer) /Copper (under layer) plating specimens.

Time (h)	Samples	*Escherichia coli* ATCC25032	*Klebsiella pneumoniae* ST101	Staphylococcus aureus 209P
0	Control	9.5×10^4 / a plate	1.71×10^5 / a plate	1.09×10^5 / a plate
24	Control	2.04×10^6 / a plate	6.76×10^6 / a plate	8.3×10^3 / a plate
	Metal plate	7.51×10^5 / a plate	1.99×10^6 / a plate	1.08×10^4 / a plate

The result in table reflected the antimicrobial characteristics of tin plating, even though the specimen had stacked single surface layers on steel substrates, since the top layer exposed to the bacteria constantly was tin at any time. As the table 2 shows, the specimens didn't show any significant antimicrobial activity against these three bacteria. The results suggest that tin plating doesn't show so drastic antimicrobial activity, being compared with copper plating.

Since the frequent used plating, tin plating, didn't have show not so high antimicrobial characteristics and the copper showed the strong antimicrobial characteristics on the other hand, we investigated the antimicrobial characteristics for the alloy plating between tin and copper to aim at the increase of antimicrobial activity of tin plating for food processing applications. For the production of the alloy plating, we used our proprietary process, Heating Stacked Single Layers Processing (HSSL Process). Table 3 shows the results for the film adhesion tests for the alloy plating. The stacked single layers specimens of tin and copper were heated at 350 degrees Celsius for three hours.

Table 3 Film Adhesion Test for the alloy plating of tin and copper produced by HSSL process.

Time (h)	Samples	*Escherichia coli* ATCC25032	*Klebsiella pneumoniae* ST101	Staphylococcus aureus 209P
0	Control	$9.5x\ 10^4$ / a plate	$1.71x\ 10^5$ / a plate	$1.09x\ 10^5$ / a plate
24	Control	$2.04x\ 10^6$ / a plate	$6.76x\ 10^6$ / a plate	$8.3x\ 10^3$ / a plate
	Metal plate	0	0	0

Table 3 indicates that the alloy plating shows the very strong antimicrobial corrosion characteristics against any of these three bacteria. The results suggest that the antimicrobial activity of tin plating would be available by alloying with copper and also that HSSL process would be an effective way to realize the proper alloying in surface layers on steel substrates.

COCLUSIONS

We investigated the antimicrobial characteristics of tin-copper plating specimens against three bacteria, *E-coli, Staphylococcus aureus, Klebsiella pneumoniae*, using slide culture tests mainly and obtained the following results.

(1) Copper plating showed high antimicrobial activities.
(2) Tin plating didn't have any significant antimicrobial characteristics.
(3) The alloy plating of tin and copper showed the strong antimicrobial activities against these bacteria.
(4) Heating Stacked Single Layers Process (HSSL Process) can be an effective method to produce antimicrobial plating on steel.

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