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**RESEARCH NOTE**

Food Science and Biotechnology



# Viability Loss of Bacteriophage MS2 Exposed to Bronze Alloy *Yugi*

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**Abstract** Cross contamination of foodborne virus via food utensils can be an important route of virus propagation. Bacteriophage MS2 was used as a surrogate for norovirus. The viability loss of bacteriophage MS2 attached to 4 kinds of metal surfaces was investigated at different temperatures and relative humidities (RH). The rate of viability loss was higher at 22°C than at 10°C and was higher at 75% RH than at 40% RH. The viability loss of the virus attached to copper or bronze surface was faster than on stainless steel or tin surface. Also the beef juice applied with the virus inoculum on the metal surfaces lowered the rate of viability loss. Although bronze was not as effective as copper in resulting the viability loss, it has been extensively used as a traditional Korean kitchen utensil and could be used more widely to decrease the viral poisoning at food processing environment and hospitals.

**Keywords:** bacteriophage MS2, survival, bronze surface, temperature, relative humidity

## Introduction

Food-poisoning viruses can be transmitted from the hands to the inanimate surfaces and vice versa (1,2). The contaminated surfaces are implicated in the transmission of virus like noroviruses (3). Virus and bacteria are able to survive on a variety of materials, however, survival rates differ on the different types of materials. Recently, the progress in the study of viral reduction have been a little achieved according to epidemically serious problem of viral food-poisoning from contaminated food by norovirus. Some studies on viral reduction using metal ion have been reported by Ditta *et al*. (4), Sjogren and Sierka (5), and Iriarte *et al*. (6).

Copper is known to have inhibitory effects on various microorganisms compared to stainless steel and plastics (7-12). Particularly, this copper is well characterized for its antiviral activity (13-16). Therefore, copper and copper-containing compounds are widely used as bactericides, algicides, fungicides, veterinary food additives, plant antibacterial agents, and for the preservation of natural and man-made materials (17,18). Also, copper bactericides have been shown to reduce phage populations *in vitro* (19). The effectiveness for biological control by using phages depends not only on the susceptibility of the target bacterium but also on environmental factors that affect phage survival (6).

In Korean dietary life, the bronze ware containing copper has been used for a long time before the advent of stainless steel ware and is used sometimes at special occasions. The bronze ware with the special composition of 78% copper and 22% tin has been called as *yugi*. There are two types of *yugi*, casted bronze ware and hammered bronze ware called *Bangza-yugi*. *Yugi* is suited to use as the table ware from the small spoons to the large tablewares because of its luster and rigidity (20). Also, Jung *et al*. (20) reported that the quantities of ppm level diffused from bronze ware would not be harmful to human health on the authority of the data. Recently, its sterilizing effect on some bacterial pathogens was also reported. In the reports by Jung *et al*. (20), Noyce *et al*. (21), and Wilks *et al*. (22), the copper alloys have higher effect for pathogen inactivation than stainless steel. The early study on the effectiveness of using the brass, a copper alloy, for door knobs in hospitals recommended its use to aid prevention of nosocomial infection. The contemporary application of stainless steel in hospital environments for work surfaces and door furniture is not recommended (23). Also, the use of the copper alloys in hospital environments may offer the potential to reduce the spread of *Clostridium difficile* infections in healthcare establishments. In addition, these increased cell death rates have been demonstrated in the previous researches on aerobic bacteria relating to food preparation surfaces (21,24,25).

Dawson *et al*. (26) and Bae and Schwab (27) used bacteriophage MS2 as a norovirus surrogate because of the culture difficulty of norovirus. Like norovirus, bacteriophage MS2 is adapted to the intestinal tract. It is a positive sense single-stranded RNA virus with icosahedral symmetry and is in the same size range at 26 nm diameter (26). Bacteriophage MS2 has been studied to help establishment of the new viral models for human pathogens and has been used previously to study sensitivity on disinfectants with a view to modeling properties of human pathogens (28-30). MS2 has been also widely used as indicator virus for disinfection efficiency (31).

When airborne microorganisms are exposed to a variety of environmental stresses, the most important ones for survival rate would be the effects of temperature and relative humidity (32). Also there has been no report on virus reduction for the bronze called *yugi*. Therefore, this paper described viability loss of virus attached to metal surfaces under the changes of temperature and relative humidity using bacteriophage MS2 as a surrogate for foodborne virus.

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## Materials and Methods

**Preparation of bacteriophage MS2** Bacteriophage MS2 from ATCC 15597-B1 was used and MS2 were assayed by double agar layer method by using *Escherichia coli* C3000 (ATCC 15597). The supernatant with cultured bacteriophage MS2 from the overnight culture of the host was got by centrifugation for 10 min at 3,000×g, and MS2 was separated by 0.2-μm syringe filter (Sartorius, Hannover, Germany), and stored at −80°C.

**Metal coupons** Four metal coupons of stainless steel, tin, copper, and bronze as copper alloy were used for exposure resistance. Stainless steel consisted of 74% Fe, 18% Cr, and 8% Sn, and a bronze of copper alloy, *yugi*, consisted of 78% Cu and 22% Sn. Tin and copper were made with only 100% Sn and Cu, respectively.

**Environmental conditions for viability loss of bacteriophage MS2** As for the environmental conditions, each metal coupon was applied to 5 different factors on temperature, relative humidity, addition of beef juice, tap water, and vegetable juice. The experiments were started at first at the temperatures of 10 and 22°C and then were conducted at the relative humidity with 40 and 75% at 22°C. Beef juice, tap water, and vegetable juice were mixed with MS2 and applied to the coupons. Beef juice from 500 g meat of beef was extracted by 10-mL of syringe and sterilized by 0.45-μm syringe filter (Sartorius). Vegetable juice (Pulmuone, Gyeonggi, Korea) was purchased at a nearby market. A 100 μL of the beef juice was add to 300 μL of bacteriophage MS2 culture, as do tap water and vegetable juice.

The metal coupons applied with 10 μL of bacteriophage MS2 solution put in the thermo-humidistat (Daihan, Seoul, Korea). Recovery was conducted by pippeting 20 times on the spots with 100 μL of distilled water for each 0, 0.5, 1, 2, 4, 8, and 24 hr exposures. The recovered solution was spread on the tryptic soy agar (TSA, BD Diagnostics, Franklin Lakes, NJ, USA), which was covered with 0.5% soft agar inoculated with host. Then plaque was counted as plaque forming unit (PFU) after incubation for 24 hr at 36±1°C. All experiments were repeated 3 times, and the mean value and standard deviation (SD) were calculated.

## Results and Discussion

**Temperature effect for viability loss of bacteriophage MS2 on metal coupons** The viability loss of bacteriophage MS2 on 4 metal coupons at different temperatures was shown in Fig. 1. It was reduced by 4-5 log PFU/mL after 8 hr on the stainless steel and the tin coupons, and by 4 log PFU/mL after 2 hr on the bronze coupon at 22°C. All MS2 were inactivated after 24 hr on the stainless steel, the tin, and the bronze coupons. However, it was reduced by 2 log PFU/mL after 0.5 hr and completely inactivated after 1 hr on the copper coupon. It was also reduced by 2 log PFU/mL on the stainless steel and the tin coupons after 8 hr at 10°C, but the remained phage kept going viability after 24 hr. However, it was reduced by 3 log PFU/mL after 24 hr on the bronze coupon. It was reduced by 3 log PFU/mL after 0.5 hr and completely inactivated after 1 hr on the copper coupon. Therefore, viability loss toward bacteriophage

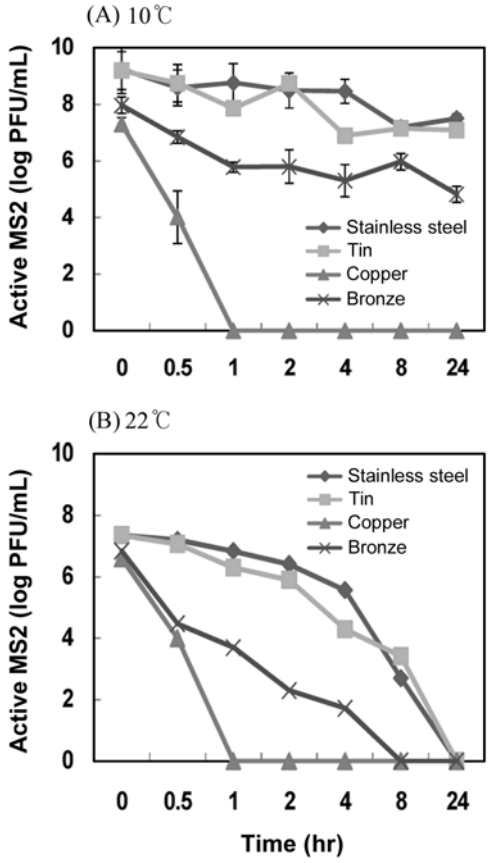


**Fig. 1. Viability loss profiles of bacteriophage MS2 on each metal coupons for 24 hr at 10 (A) and 22°C (B) without relative humidity control.** *Yugi* was used as a bronze coupon.

MS2 was faster at 22°C than at 10°C. Copper and bronze showed the better effects than stainless steel and tin. Ditta *et al*. (1) reported that thin films of $TiO_2$ were anti-viral and inclusion of copper increased the rate of inactivation, and these surfaces might be applied in infection control. Mehtar *et al*. (33) also reported that copper, copper alloy, and nickel silver inhibited nosocomial pathogens at maximum 6 hr, on the other hands, stainless steel and PVC showed little or no inhibitory activity. Jung *et al*. (20) again reported that copper alloy did not showed inactivation effect dramatically as copper, but it showed the effect gradually according to the conditions. Weaver *et al*. (24) reported that when compared with stainless steel, a significant increase in cell death rate could be seen on the surfaces of the copper alloy with above 70% copper.

**RH effect for viability loss of bacteriophage MS2 on metal coupons** The viability loss effect toward bacteriophage MS2 on 4 metal coupons by relative humidity at 22°C was shown in Fig. 2. At RH 40%, it was reduced by 3 log PFU/mL on the stainless steel and the tin coupons after 8 hr, but the remained phages keep going viability after 24 hr. The bronze coupons inactivated by 5 log PFU/mL after 8 hr, whereas the alive phages kept going viability after 24 hr. It was reduced by 6 log PFU/mL on the copper coupon after 0.5 hr and completely inactivated only after 1 hr. At RH 75%, it was reduced by 4 log PFU/mL on the stainless steel

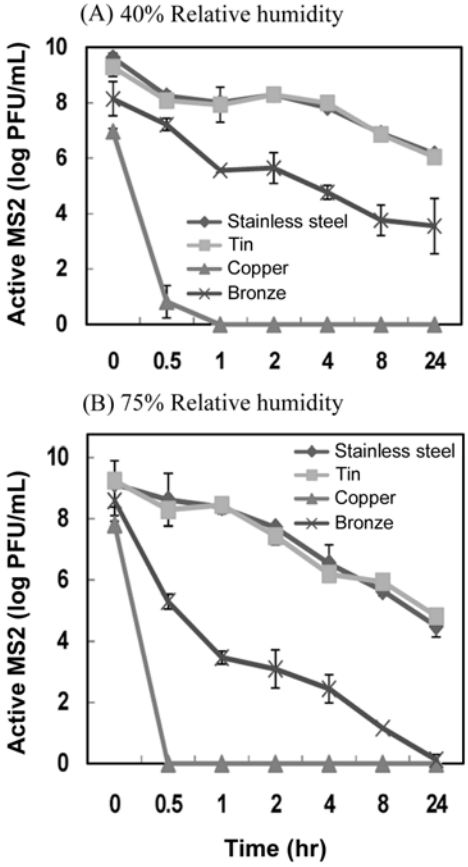


**Fig. 2. Viability loss profiles of bacteriophage MS2 on each metal coupons for 24 hr at the relative humidities of 40 (A) and 75% (B) at 22°C.** *Yugi* was used as a bronze coupon.

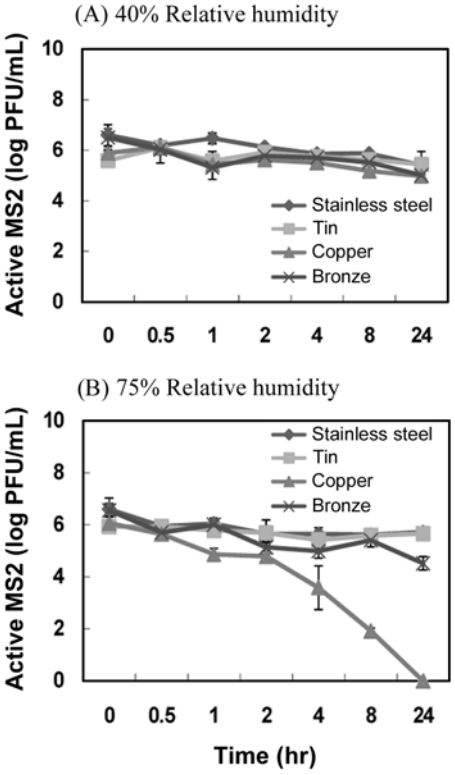


**Fig. 3. Viability loss profiles of bacteriophage MS2 with beef juice on each metal coupons for 24 hr at the relative humanities of 40 (A) and 75% (B) at 22°C.** *Yugi* was used as a bronze coupon.

and the tin coupons after 8 hr and reduced by 5 log PFU/mL after 24 hr. As for on the bonze, it was reduced by 7 log PFU/mL after 8 hr and showed viability loss effect gradually by 8 log PFU/mL after 24 hr. However, on the copper coupon, it was reduced by over 6 log PFU/mL after 0.5 hr. Mbithi *et al.* (34) observed that virus survived better at the lower RH levels than at the high or ultra high RH. Also, Tseng and Li (35) reported that the susceptibility for bacteriophage MS2 was higher at 85% RH than that at 55% RH. Therefore, bacteriophage MS2 seemed to be inactivated more easily at high relative humidity on copper and bronze copper alloy. Finally, viability loss of bacteriophage MS2 was efficient at 22°C than at 10°C, and it was also better at RH 75% than at 40%. Though the effects of temperature and RH varied according to attached metal, there were higher inactivating effects in case of high temperature and relative humidity for any metal types.

**Beef juice, tap water, and vegetable juice effect for viability loss of bacteriophage MS2 on metal coupons**

To understand the influence of food component for inactivation, the beef juice, tap water, and vegetable juice were used as a model system. After being mixed with the beef juice, it almost showed no viability loss effect for all the metal surfaces at RH 40% and 22°C (Fig. 3). At RH 75%, there were no viability loss activities on the stainless steel and the tin coupons, whereas it was reduced by 2 log PFU/mL on the bronze coupon after 24 hr. It was also reduced by 5 log PFU/mL on the copper coupon after 8 hr and completely inactivated after 24 hr. After being mixed with the tap water, it almost showed no viability loss effect for all the metal surfaces except for copper coupon at RH 40% and 22°C (Fig. 4). On the copper coupon, it completely inactivated after 2 hr. At RH 75%, there were no viability loss activities on the stainless steel and the tin coupons, whereas it was reduced by 2 log PFU/mL on the bronze coupon after 24 hr. It was also reduced by 2 log PFU/mL on the copper coupon after 0.5 hr and completely inactivated after 1 hr. After being mixed with the vegetable juice, it showed no viability loss effect for stainless steel and the tin coupons regardless of RH at 22°C (Fig. 5). Whereas it was reduced by 4-5 log PFU/mL on the bronze coupon after 24 hr and completely inactivated after 1 hr on the copper coupon regardless of RH at 22°C. Noyce *et al.* (21) observed that the beef extract itself might provide a protective matrix for the bacterial cells to "hide in" from the detrimental effects of copper exposure and this might be due to fat content. So bacteriophage MS2 mixed up with the extract would be also protected from the copper exposure. In case of metal surface, stainless steel and tin had a little effect on bacteriophage MS2, the other side copper might have an effect on bacteriophage MS2 as characteristic of strong adhesion.

Treatment with copper and silver has been reported to be effective for the removal of bacteriophages and poliovirus form water (36,37). Abad *et al.* (38) also reported that

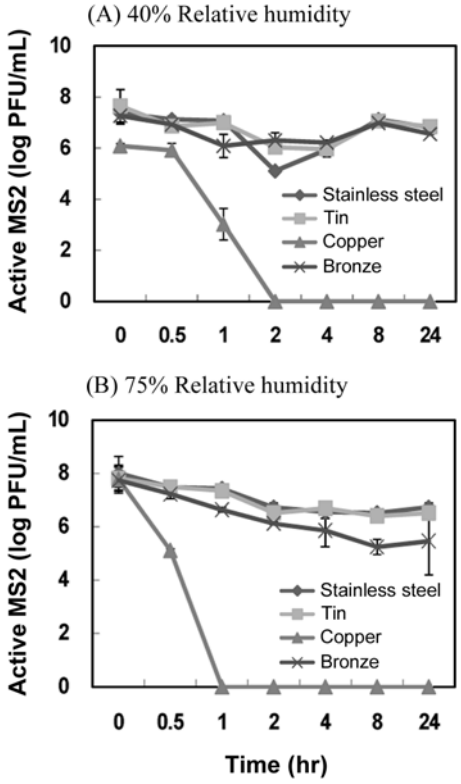


**Fig. 4. Viability loss profiles of bacteriophage MS2 with tap water on each metal coupons for 24 hr at the relative humanities of 40 (A) and 75% (B) at 22°C.** *Yugi* was used as a bronze coupon.

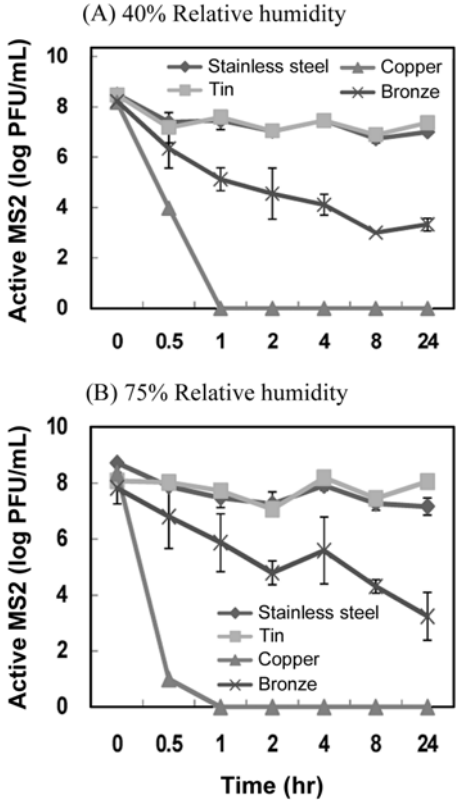


**Fig. 5. Viability loss profiles of bacteriophage MS2 with vegetable juice on each metal coupons for 24 hr at the relative humanities of 40 (A) and 75% (B) at 22°C.** *Yugi* was used as a bronze coupon.

poliovirus showed more than a 4 log titer reduction in the presence of copper and silver combined with free chlorine. Mehtar *et al*. (33) reported that copper and its alloys demonstrated good antimicrobial activity against multiple-antibiotic-resistant nosocomial bacteria. Stainless steel was widely used in the healthcare environment because it was corrosion resistant to most cleaning materials and always appeared clean, but there was no inherent antimicrobial advantage to using this metal.

Therefore, copper and bronze showed viability loss effect toward this virus especially in case of high temperature and relative humidity. Particularly the bronze of *yugi*, a copper alloy, has been used in Korea because of strongness unlike copper. Copper is not suitable as materials unlike stainless steel because it is soft and not durable. Henceforth, even though copper showed more effective for antiviral activity than bronze, some contributions to viability loss of food-poisoning virus by direct contact between metal surface and virus might be achieved provided the bronze of *yugi*, a copper alloy, apply to the hospital, the public facilities, and the food-processing environments.

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