J. Trop. Agric. and Fd. Sc. 34(2)(2006): 303–308

Biological disinfection of specific contaminating bacteria on stainless steel food preparation surface

(Pembasmian kuman secara biologikal terhadap bakteria kontaminasi spesifik di atas permukaan penyediaan makanan keluli tahan karat)

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Key words: biological disinfection, *Escherichia coli*, stainless steel food preparation surface

Abstract

A study of the possibility of using bacteriophage as a disinfecting agent for the control of contaminating bacteria on food preparation surface was conducted. *Escherichia coli* strain K12 and T4 bacteriophages were used as bacteria and bacteriophage disinfecting model. Stainless steel food contact surfaces were used in the experiments. Survival of bacteria without disinfection and survival of bacteria disinfected with 1% and 10% bacteriophage suspension was investigated. A 10% bacteriophage suspension appeared to be effective in reducing the number of the test organism *(Escherichia coli)* on the contact surface, as the number of surviving bacteria on the test surface was generally lower at a shorter contact time as compared to 1% bacteriophage suspension. The rate of disinfecting ability of bacteriophage was shown to be a function of the number of contaminating bacteria on a surface. Heavy surface contamination requires more time to be disinfected. The results indicated that bacteriophage in the form of suspension was applicable for the control of specific contaminating bacteria on the tested food contact surface.

Introduction

Food contamination is a major concern in the food industry and may be considered as the occurrence of any objectionable matter in or on the food. Food may be contaminated with faecal materials, spoilage or food poisoning bacteria either from bacteria or part of the processing. Adhesion of microorganism to food processing equipment and surfaces and the problem it causes is a matter of concern to the food industry (Barnes 1999). To prevent such occurrences, contamination must be kept to a minimum. Sprenger (2002) described three types of contamination namely microorganism, physical, and chemical.

Significant bacteria contamination of pathogens results in large amount of spoilt food and unacceptable numbers of food poisoning cases. Sometimes bacteria are passed directly from the source to high risk food, but as bacteria are largely static and as the sources are not always in direct contact with food, bacteria have to rely on other things to be transferred to food. These things are called vehicles and the main ones are

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hands, hand-contact surfaces, cloth and equipment, and food contact surfaces. The path along which bacteria are transferred from the sources to food can be disrupted by cleaning and disinfection. Disinfecting by chemical means has been used widely and extensively in the food industry. However, there have been some pros and cons of its effectiveness, environmental effects as well as cost.

A totally new approach is being considered to combat contaminating bacteria on food contact surfaces by biocontrol methods. This involves the application of a bacterial virus i.e. bacteriophage that will kill only specific bacteria.

Surprisingly, although bacteriophage has been studied intensively in molecular genetic, very little work has been reported on the application of bacteriophages as a biological control. Few researchers have reported the usage of bacteriophages as biocontrol on fruit and vegetable (Anon. 2002) but none has been cited for the application on food contact surfaces. The objective of the study was to evaluate the ability of bacteriophage to eradicate pathogenic bacteria from test surface which would normally be used for food preparation.

Materials and methods

Test organism

The test organism used is *Escherichia coli* strain K12 (W3110) obtained from the University of Hertfordshire, United Kingdom. The bacteria was kept in a stabbed culture at room temperature.

Maintenance of bacteria culture

The *E. coli* bacteria from the stabbed culture (stock culture) was subcultured onto nutrient agar and incubated at 37 ºC on weekly basis (Roberts et al. 1995).

Preparation of overnight culture

The *E. coli* colony from the nutrient agar was inoculated into 500 ml sterile nutrient broth and incubated overnight at 37 ºC in an orbital shaker at 100 rpm. The prepared overnight culture was then used for the experiment (Williams, R., Univ. of Lincoln. pers. comm. 2003).

Bacteriophage lysate stock

The bacteriophage used was *E. coli* T4 strain G1T1 obtained from the University of Hertfordshire, United Kingdom. The bacteriophage lysate stock was kept in a 1.5 ml sterile plastic ampoule at room temperature.

Preparation of T4 bacteriophage lysate

Overnight *E. coli* culture, strain K12 (W3110) was grown in 100 ml phage broth in a 250 ml conical flask at 37 ºC in an orbital shaker. On the following day, 5 ml of *E. coli* overnight culture was inoculated into a 100 ml of phage broth at 37 ºC in an orbital shaker. A second flask was prepared similarly as control. After 1.5–2 h of moderate turbidity growth, the broth was ready to be sampled for optical density at 550 nm. Approximately 1 ml of phage lysate stock was added to one of the flasks. The other flask (control) was not added with phage. A 3 ml volume of the broth was transferred to a silica cuvette and the reading was recorded using spectrophotometer model UV-1601 Shimadzu.

Figures 1–2 show the optical density reading for both the control and the treated samples. After clearing of bacteria (i.e. when the optical density reading was constant as shown in *Figure 2*), 5 ml of chloroform was added and left to be settled, after which the chloroform was decanted and the resulting lysate was stored at room temperature in a sterile 100 ml bottle. The volume of the T4 bacteriophage lysate recovered was approximately 70–75 ml. and the number of bacteriophage in the lysate was approximately 10^{10} pfu/ml (plague forming unit per ml) as determined by phage assay technique (Williams, R., Univ. of Lincoln, pers. comm. 2003).

Preparation of bacteriophage spray suspension

A 10-fold suspension of bacteriophage lysate was prepared by diluting 10 ml of the bacteriophage lysate with 90 ml sterile phage buffer (pH 7.0) in a spray container. This preparation is equivalent to 10% bacteriophage suspension. The spray container was rinsed with boiling distilled water prior to filling. A 100 ml measuring cylinder rinsed with boiled distilled water was used for the measurement of phage buffer. Similarly a 100-fold suspension of bacteriophage lysate, which is equivalent to 1% bacteriophage suspension, was prepared by diluting 1 ml of bacteriophage lysate with 99 ml phage buffer. Fresh spray suspension was used each time the experiments were carried out (Dixon, R., University of Lincoln, pers. comm. 2003).

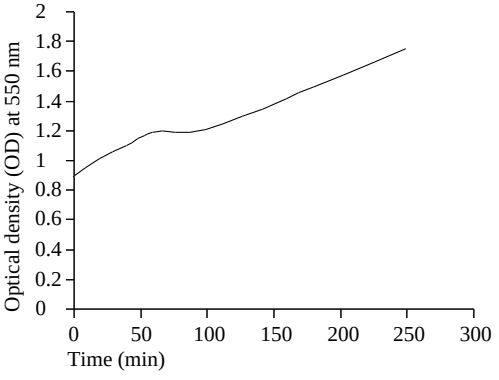

*Figure 1. Growth of **Escherichia coli** in broth culture without T4 bacteriophage, (control) showing the normal growth curve of the bacteria. The optical density represents the concentration of the bacteria*

Artificial contamination of test surface

Stainless steel surface plates (30 cm x 40 cm) were artificially contaminated with overnight *E. coli* bacteria. A 2% sterilized gelatin solution was used as sticky substance (carrier) and poured onto the test surface. When the gelatin solution was dry, the overnight bacteria culture was poured and left to adhere onto the sticky substance on the test surfaces. Bacteriophage suspension of 1% and 10% concentration were sprayed onto the surfaces. Sampling was done by swabbing; before, and after 2 h and 4 h of spraying.

Experiments on the survival of the bacteria without disinfection were done under the same procedure, except the phages were not included in the spray suspension. In an additional experiment, bacteriophage

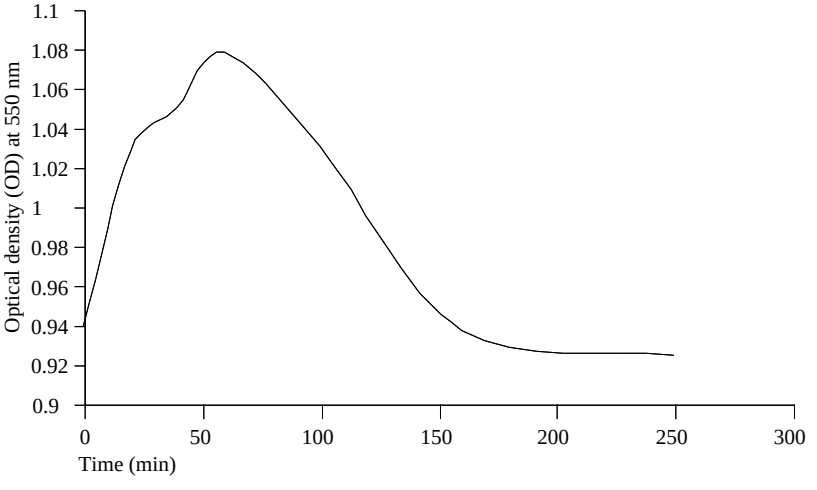

*Figure 2. Growth of **Escherichia coli** in broth culture treated with T4 bacteriophages, showing lysing activity of the phage. The bacteria number started to decrease after 60 min of bacteriophage addition. The resulting clear broth was used as bacteriophage lysate which was then diluted accordingly to make bacteriophage suspension*

suspension was sprayed onto surface contaminated with different concentration of *E. coli* bacteria to study the effect of bacteriophage number on the removal of amounts of contaminating bacteria. All experiments were done at ambient temperature (23 ± 2 °C) (Dixon, R., Univ. of Lincoln, pers. comm. 2003).

Recovery of bacteria from test surface

Sampling was done by swab method (Roberts et al. 1995). An approximate area of 25 cm^2 was used for sampling. The area was swabbed using standard throat swab previously moistened with sterile Ringers solution. Enumeration of the bactera survival was done by a 10-fold serial dilution of the sample followed by standard spread plating technique using 2 replicates. Plates were incubated at 37 ºC for 48 h. Results were expressed in cfu/ml (colony forming unit per ml).

Results and discussion

Survival of bacteria on test surfaces without disinfection

The number of *E. coli* bacteria was reduced on the test surfaces throughout the contact time *(Table 1)* indicating that under normal condition, environmental factor such as surface drying also affected the growth of bacteria.

Survival of bacteria on test surfaces disinfected with T4 bacteriophage

In the experiment using 1% T4 bacteriophage suspension *(Table 1)*, the initial number of the *E. coli* bacteria was reduced from 10^5–10^6 cfu/ml to 10^4 cfu/ml after 2 h, and was further reduced to 0 and 10^3 cfu/ml after 4 h of spraying. The reduction in the number of bacteria as indicated after 2 h could be the result of the bacteriophage lysing activity.

In the 10% T4 bacteriophage suspension experiment *(Table 1)*, the initial number of bacteria recovered from the surface points was 10^5–10^6 cfu/ml. After 2 h of bacteriophage application, 10^3 cfu/ml of the bacteria was recovered. The survival of the bacteria at this stage was shown to be lower than using 1% bacteriophage suspension. The *E. coli* bacteria were not detected (i.e. zero survival) after 4 h of the spraying process. Therefore, a total removal or 100% reduction of bacteria can be said to be achieved after 4 h of bacteriophage spraying. A 10% T4 bacteriophage suspension which contained approximately 10^9 plaque forming unit per ml (pfu/ml) of bacteriophages (i.e. 10-fold dilution of the original number) could therefore completely eradicate the *E. coli* bacteria after 4 h of bacteriophage application. The number of bacteria survived was also lower than those survived in the experiment without

Table 1. Survival of *Escherichia coli* (cfu/ml) on stainless steel surface without disinfection, disinfected with 1% and 10% T4 bacteriophage suspension

Sampling point* (random)	Initial number of bacteria on surface	Number of bacteria after 2 h	Number of bacteria after 4 h
Without disinfection			
1	10^6	0	10^3
2	10^6	10^6	10^3
Disinfected with 1% T4 bacteriophage suspension			
1	10^5	10^4	10^3
2	10^6	10^4	0
Disinfected with 10% T4 bacteriophage suspension			
1	10^5	10^3	0
2	10^6	10^3	0

disinfection, indicating that the lysis process had taken place. The rate of bacteria reduction was comparatively better using a 10% bacteriophage suspension than 1% as the number of bacteria survived was generally lower at a shorter contact time.

Higher concentrations of the bacteriophage suspension was shown to have resulted in a lower survival number of bacteria at a shorter contact time. The bacteria reduction was greater when the bacteriophage spray suspension was used, particularly at a higher concentration, as compared to without bacteriophage disinfection, thus indicating that the disinfecting effect by the phage was taking place. Based on the lytic cycle of the bacteriophage, the phages multiplied and infected all the bacteria until none of them survived. This could be effectively achieved after 4 h of spraying using 10% bacteriophage suspension. Hadas et al. (1997) found that T4 bacteriophage adsorption rate is dependable on the *E. coli* bacterial growth rate. Faster adsorption rate was reported at a higher bacterial growth rate. Parameters for the phage development and cell lysis were also reported to be bacterial growth dependent (Hadas et al. 1997).

Surrounding temperature may affect the relative humidity thus affecting the drying of the surface. The bacteria will die slowly under such condition. This process may acquire a longer period. The dying process of bacteria, however, is accelerated if an antimicrobial agent such as bacteriophage is applied. Bacteriophage can reduce the number of bacteria on surface to a minimum after certain period of contact. Chemical and physical parameters play an important part for the effectiveness of any disinfecting agents, and they must be chosen accordingly as to produce desirable and satisfactory results.

Effects of T4 bacteriophage on different concentrations of bacteria on stainless steel food contact surface

An additional experiment to determine the effect of bacteriophage on different concentrations of *E. coli* bacteria on food contact surface was done. A 10% bacteriophage suspension was used in the experiment. The 10^{-3} and 10^{-6} tenfold serial dilution of an *E. coli* overnight culture were used as the contamination levels. The *E. coli* overnight culture contains 10^{8} cfu/ml which means that a 10^{-3} and a 10^{-6} dilution contain approximately 10^{5} and 10^{2} cfu/ml respectively.

The number of bacteria in 10^{-3} dilution of *E. coli* was reduced to not more than 10^{4} cfu/ml after 2 h of T4 bacteriophage spraying and the number decreased to 0 after 4 h which was a total removal or 100% reduction of the bacteria *(Table 2)*.

The rate of reduction was faster when applying bacteriophages on lower concentration of bacteria as shown in the experiment using 10^{-6} bacteria dilution *(Table 2)*. There was no growth of bacteria after 2 h, whereas when using a 10^{-3} overnight culture, some bacteria were

Table 2. Survival of 10^{-3} and 10^{-6} dilution of *Escherichia coli* overnight culture (cfu/ml) on stainless steel disinfected with 10% T4 bacteriophage spray suspension

Sampling point* (random)	Number of bacteria sprayed onto surface	Number of bacteria after 2 h sprayed with phages	Number of bacteria after 4 h sprayed with phages
10^{-3} dilution			
1	10^{5}	$< 10^{3}$	0
2	10^{5}	$< 10^{4}$	0
10^{-6} dilution			
1	10^{2}	0	0
2	10^{2}	0	0

detected. The effect of the number of bacteria on the test surface has been demonstrated in the experiment. Therefore, it can be said that the reduction of the bacteria on contaminated surface by the phage is as well the function of the number of bacteria initially present on the surface. The time required for the eradication of bacteria was found to be at a faster rate when applying the bacteriophages on surface with lesser number of contaminating bacteria. Heavily contaminated surfaces therefore require more time for bacteria removal.

Conclusion

The study provided some information on the application of bacteriophage for the eradication or removal of specific bacteria on food preparation surfaces. Under natural conditions such as drying by air, bacteria are less able to survive on the contact surface. The bacteriophage suspension prepared in this study was relatively effective at pH approximately 7.0 which was the pH of the buffer used for the suspension. The outcome of the study also showed that higher numbers of bacteriophage eradicated more bacteria than at a lower concentration. Generally, bacteriophage could therefore be useful for eradication of bacteria on stainless steel food contact surface.

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Abstrak

Kajian kemungkinan menggunakan bacteriofaj sebagai agen pembasmi kuman untuk kawalan bakteria kontaminasi di atas permukaan penyediaan makanan telah dibuat. Bakteria *Escherichia coli* strain K12 dan bakteriofaj T4 digunakan sebagai model. Permukaan penyediaan makanan jenis keluli tahan karat digunakan dalam eksperimen ini. Kadar pertumbuhan bakteria bagi permukaan tanpa basmi kuman, dibasmi kuman dengan 1% dan 10% suspensi bakteriofaj dikaji. Larutan suspensi 10% bakteriofaj didapati lebih berkesan untuk membasmi bakteria *(E. coli)* di atas permukaan yang diuji, dengan jumlah bakteria yang hidup di atas permukaan adalah lebih rendah dalam jangka masa pendek berbanding dengan larutan suspensi 1%. Kadar pembasmian kuman bakteriofaj bergantung kepada jumlah bakteria kontaminasi. Permukaan yang mempunyai aras kontaminasi yang tinggi memerlukan jangka masa pembasmian yang lebih lama. Keputusan menunjukkan larutan suspensi bakteriofaj boleh digunakan untuk mengawal bakteria kontaminasi spesifik di atas permukaan yang diuji.

Accepted for publication on 5 April 2006