REMOVAL OF ESCHERICHIA COLI O157:H7 ON BEEF BY BACTERIOPHAGE

YUTAKA MORIOKA, MASAHIRO NUMATA, TOYOO NAKAMURA, SAYURI YAMAGUCHI*,and SEISHI TAKAHASHI*

ITOHAM Central Research Institute, 1-2 Kubogaoka, Moriya, Kitasouma, Ibaraki 302-0104, Japan
* Nagaoka National College of Technology, 888 Nishikatakai, Nagaoka, Niigata 940-0817, Japan

BACKGROUND

Escherichia coli O157:H7 has become a pathogen of primary concern to the food industries since documentation of its association with several serious outbreak of food born illness (Mermelistein 1993). It is often pointed out that the intestinal tract of cattle is an important reservoir of *E.coli* O157:H7. Raw food of bovine origin through fecal contamination during slaughter are likely to be vehicles of *E.coli* O157:H7. The slaughter and processing carcasses are important control point for the prevention of *E.coli* O157:H7 infection (Doyle 1991).

Developed new effective chemical methods and advanced techniques to minimize the pathogenic bacteria on carcasses have been reported. Carcass washing with organic acid solutions or hot water is one method used currently to decontaminate microbial contamination.

On the other hand, group of Prof.Takahashi to Nagaoka National College of Technology has isolated bacteriophage which lyse effectively *E.coli* O157:H7. To the best of our knowledge, there is so far no study of bacteriophage which remove *E.coli* O157:H7 on beef.

OBJECTIVE

The objective of this study was to confirm lysis effect of bacteriophage for *E.coli* O157:H7 on beef.

METHODS

Meat sample

Beef outside (thickness:5mm, 5×5cm^2) was used in this study.

Strain used

For easy detection from beef sample Ampicillin and Streptomycin resistance *E.coli* O157:H7 strain was used in this study.

Bacteriophage used

Bacteriophage strain used in this study lysis for *E.coli* O157:H7. They have been isolated from several samples from environment.

Preparation of inoculum

E.coli O157:H7 was cultured in L-broth (Peptone 10g, Yeast extract 3g, NaCl 2.5g, 20%Glucose solution 5ml, 20%Maltose solution 5ml, 1M $CaCl_2$ solution 1ml, Distilled water 1000ml) at 37 ℃ on a shaker to give the turbidity reading of 0.2 on spectrophotometer at 600nm. The inoculum was decimally diluted with L-broth for 10^1 and 10^2 cfu/ml.

Preparation of bacteriophage suspension

Bacteriophage used in this study was prepared by lysis procedure on *E.coli* O157:H7 in L-broth at 37 ℃ . Phage suspension were made bacteriologically sterile by membrane filtration (0.2μm porosity) and stored at 4 ℃ until use.

Inoculation of *E.coli* O157:H7

Each beef samples was inoculated with 1ml concentrated *E.coli* O157:H7 which resulted in 2.0×10^1 and 2.0×10^2 cfu/beef.

Addition of bacteriophage

Bacteriophage suspension was added to 1ml which resulted in 7.8×10^8/beef for each beef sample. Control was not added bacteriophage suspension.

Storage temperature and times

After inoculation of *E.coli* O157:H7 and addition of bacteriophage, beef samples were stored at 10, 25 and 37 ℃ (respectively refrigeration, room and hot carcass temperature) and 1, 2 and 3 hours.

Enumeration of surviving *E.coli* O157:H7

After storage, beef samples were enumerated surviving *E.coli* O157:H7 with L-agar (L-broth with agar 1.5%, Ampicillin 50μg/ml and Streptomycin 25μg/ml final). For determination of surviving *E.coli* O157:H7, L-broth 50ml was combined with each beef sample and shook for 1min. Surviving *E.coli* O157:H7 in shook L-broth 50 ml were harvested by centrifugation at 4500 rpm for 10 min and suspended L-broth. Pour plate technique was used for surviving *E.coli* O157:H7 count and incubated at 37 ℃ for overnight.

RESULT AND DISCUSSION

Figure 1 and 2 shows the surviving number of *E.coli* O157:H7 after storage at 37 ℃ .

When stored at 37 ℃ , both inoculation of 10^1 and 10^2 *E.coli* O157:H7 increased than initial number (Phage −). Addition of phage (Phage +) could decrease surviving *E.coli* O157:H7. Storage at 25 ℃ (Figure 3 and 4) and 10 ℃ (Figure 5 and 6) were also same results. Figure 5 show the result 10^1 inoculation of *E.coli* O157:H7 and storage at 10 ℃ . When stored for 3 hours and phage added sample was not detected *E.coli* O157:H7. Accordingly, storage time extension gives suggestion to perfectly lysis effect on meat.

The storage temperature had no effect the tendency to decrease *E.coli* O157:H7 in this study.

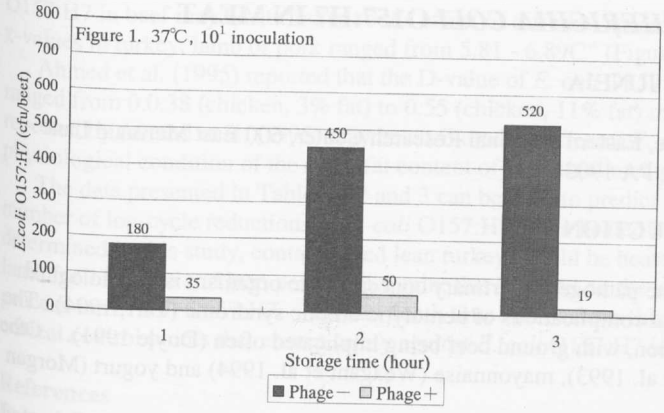
Bacteriophage used in this study has lysed for *E.coli* O157:H7 number on meat. However, the effect was not perfectly. We suggested some factor has inhibited effect of phage. Further study to confirm lysis effect of bacteriophage against *E.coli* O157:H7 is needed.

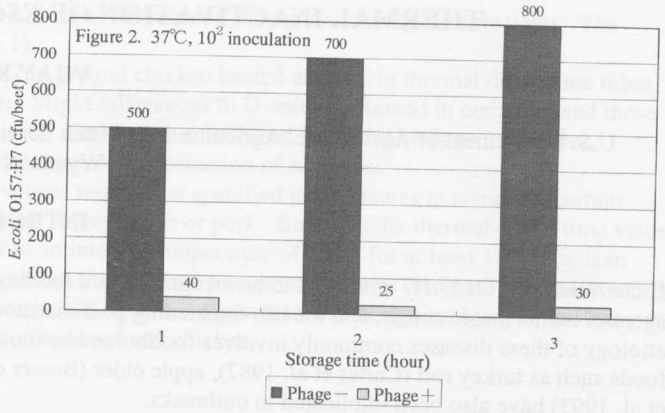
CONCLUSIONS

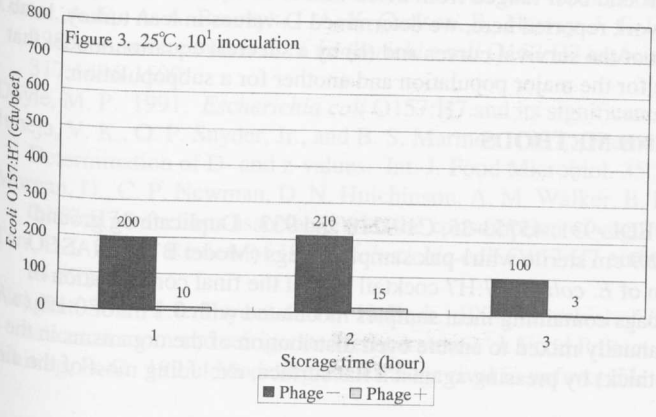
Bacteriophage effectively inhibited growth and decrease *E.coli* O157:H7 strain in this study on beef stored at all temperature. However, the effect was not perfectly. Further study to confirm lysis effect is needed.

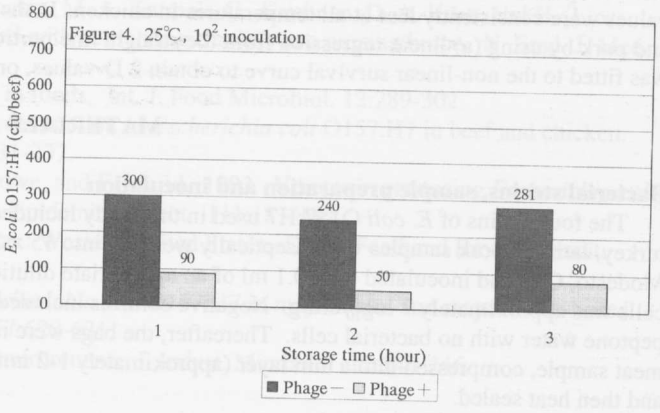
REFERENCE

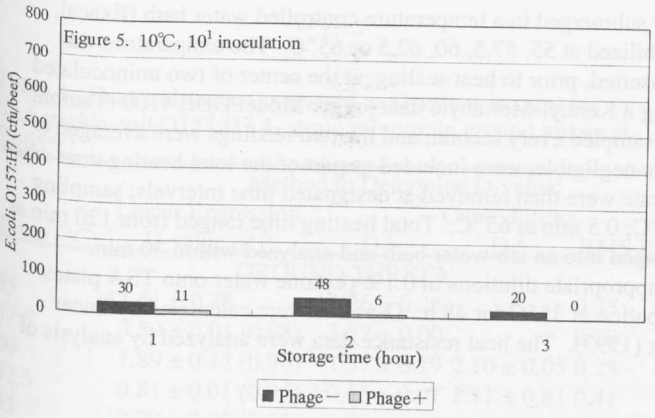
Michael P. Doyle. 1991 *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289-301.
Mermelstein, N. H. 1993. Contorolling *Escherichia coli* O157:H7 in meat. Food Technol. 47(4): 90-91.











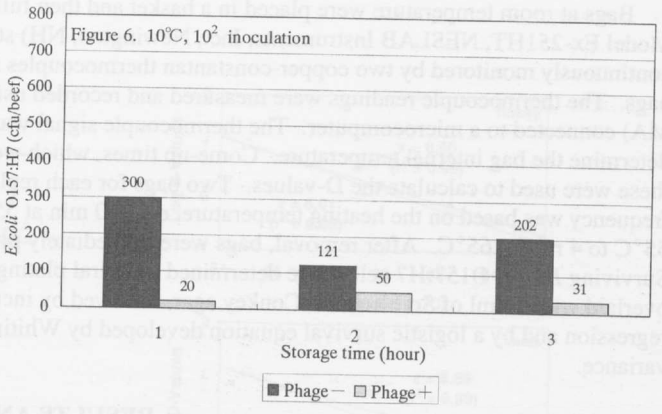


Figure 1 ~ 6 Surviving number of *E.coli* O157:H7 after storage. (Phage − : phage not added , Phage + : phage added)