Fayoum University
Faculty of Science
Botany Department

Application of bacteriophage to control some contaminating bacteria in meat samples

By

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English summary

Food contamination is a serious issue because it results in foodborne diseases. It can be microbial or environmental. Meat is a good medium for the multiplication of bacteria that cause food-borne diseases. Control of those food-borne pathogens has been done using various natural or chemical food preservatives. Chemical preservatives are not preferred due to the side effects they cause.

Bacteriophages are viruses that used as biocontrol and bio preservation agents. They are really effective and specific against their bacterial host without a side effect on the intestinal microflora.

This study aims to:

Isolation of bacteria from twenty samples of fresh meat and meat products (fresh meat, burgers, Luncheon, sausage and pastrami). Large number of bacterial species were isolated and counted on nutrient agar.

S. aureus, *E. coli* and *K. pneumoniae* were the most common pathogens in the samples. Three pathogens were cultured on plates of EMB, XLD and MAS media. The pathogenic bacteria were appeared variability in growth rate.

Bacterial isolates were confirmed morphologically by their growth on solid selective medium, investigated microscopically by gram staining and some biochemical characteristics. Bacterial isolates were identified by using VITEK II automated system which confirmed that they were *Staphylococcus aureus*, *Klebsiella pneumoniae ssp pneumoniae and Escherichia coli.*

Examination the sensitivity of isolated bacteria against thirteen antibiotcs (Ceftriaxone, Tigecycline, Gentamicin, Rifampicin, *cefoxitin,* Ciprofloxacin, Ampicillin, Penicillin, Erythromycin, Fusidic Acid, Vancomycin, Tetracycline and Clindamycin). *S. aureus* isolate showed sensitivity for all antibiotics Except for Ampicillin and Penicillin. *E. coli* isolate showed sensitivity for Ceftriaxone, Tigecycline, Gentamicin, Rifampicin, *cefoxitin* and Ciprofloxacin but resistant to Ampicillin, Penicillin, Erythromycin, Fusidic Acid, Vancomycin,

Tetracycline and Clindamycin. *K. pneumoniae* isolate showed sensitivity for Gentamicin, Tetracycline and Cefoxitin but resistant to Ampicillin, Tigecycline, Penicillin, Erythromycin, Fusidic Acid, Vancomycin, Rifampicin, Ceftriaxone, Ciprofloxacin and Clindamycin. *E. coli* and *K. pneumoniae* were resistant isolates as they resist to more than antibiotics related to three different classes.

The phage suspension was prepared from different collected sewage water samples by centrifugation. The suspension then added to the broth media containing overnight *S. aureus*, *E. coli* and *K. pneumonia* and incubation. The phages were detected in the suspension with spot test and double agar layer (DAL) method.

Only one *S. aureus* phage, two *E. coli* phages and one *K. pneumoniae* phage were isolated by Single plaque isolation which was repeated for three times to obtain biologically purified phages. The phages were isolated depending upon the plaque morphology.

The morphological plaque properties of *S. aureus* phage had clear circular plaque of 1.5-2 mm in diameter, *E. coli* phage1 had clear circular plaque of 2 mm in diameter but *E. coli* phage2 had turbid circular plaque of 2.5 mm in diameter with halo and *K. pneumoniae* phage had clear circular plaque of 6.0 mm in diameter with large halo.

Titers of phages were estimated after propagation and achieved 2.6×10^{9} PFU/mL for *S. aureus* , 3×10^{10} PFU/mL for *E.coli* phage1, 1.4×10^{10} PFU/mL for *E.coli* phage2 and for 2×10^{9} PFU/mL *K. pneumoniae* phage.

TEM micrographs for *S. aureus* phage particle which has contractile tail with 153nm in length, Isometric head with 58 nm in size and belonged to *Myoviridae* family, *E. coli* phage 1 particle has non contractile tail with 184nm in length, Oval head with 47 nm in size and belonged to *Siphoviridae* family *E. coli* phage 2 showed that the particle has non contractile tail with 183.5 nm in length, Hexagonal head with 83nm in size and belonged to *Siphoviridae* and *K.*

pneumoniae phage particle has no tail, icosahedral head with 69.2 nm in size and belonged to *Podoviridae* family.

Four isolated phages were exposed to different pH values (4, 5, 6, 7, 8, 9, 10, 11and 12). *S. aureus p*hage was tolerant to (4 - 10) pH*; E. coli* phage1 was tolerant to (4 - 9) pH but *E. coli* phage was tolerant until pH 11 and *K. pneumoniae phage* was tolerant to (5-10) pH for 24 h of incubation period.

Four isolated phages were exposed to four different concentrations of NaCl solution (5%, 10%, 15% and 20%) and different concentrations of spices (0.5%, 1%, 3% and 5%). The phages were tolerant to all concentrations of NaCl solution and spices concentrations.

Four isolated phages were exposed to UV light up to 40 cm for different periods of time (5, 10, 15 and 20 min). The phages were tolerant to all periods of exposure.

The dilution end point was achieved with Spot test, DEP 10^{-7} for *S. aureus* phage, 10^{-9} for *E. coli* phage1, 10^{-8} for *E. coli* phage2 and 10^{-6} for K. *pneumoniae* phage.

Phages were exposed to different temperatures (20^{o}C, 30^{o}C, 35^{o}C, 37^{o}C, 38^{o}C, 39^{o}C, 40^{o}C, 45^{o}C, 50^{o}C, 60^{o}Cand 70^{o}C). The thermal inactivation point for each phage was determined It was 50°C for *S. aureus* phage, while it was 45°C for *E. coli* phage1, 2 and it was 50°C for *K. pneumoniae* phage.

The host range of phages was determined by spot test. *S. aureus* phage was tested against seven bacterial isolates belonging to *staphylococcus spp*, *E. coli* and two *salmonella* and it could lyse all the tested bacterial isolates except *E.coli* and *salmonella* isolates. *E. coli* phages were tested against four bacterial isolates belonging to *E. coli* and *salmonella spp* and they were capable of causing lysis of all the tested isolates except *salmonella* spp. *K. pneumoniae* phage was tested against Four bacterial isolates belonging to *Klebsiella* spp and two *E. coli* isolates and it couldn't lyse all the Tested bacterial isolates.

Phages were preserved at three different temperatures (room temperature, 4°C and -20°C). They could maintain their viability for more than two months, the titer of phages was reduced gradually by the time but with very low values. The results showed that - 20□C was the best degree for long preservation for phages, 4°C also a good temperature for preservation and the sample preserved at room temperature showed the least concentration of phages after about nine weeks of preservation.

An applied experiment was designed to use cocktail with MOI 100 from *S. aureus, K. pneumoniae* and *E. coli* phages to reduce these microbes' numbers in fresh meat at room temperature and at 4°C.

There are three treatments at room temperature:

1. Ten g of non-sterilized fresh meat was left without any treatment after estimation the *S. aureus*, *K. pneumoniae* and *E. coli* bacteria on it.
2. Ten g of non-sterilized fresh meat was treated with 1 mL of phage cocktail (1:1:1 of 10^{10} PFU/mL of each *S. aureus*, *K. pneumoniae* and *E. coli* phages).
3. Ten g of non-sterilized fresh meat was treated with 1 mL of phage cocktail (1:1:1 of 10^{10} PFU/mL of each *S. aureus*, *K. pneumoniae* and *E. coli* phages) followed by addition 1 g spices.

- Aliquots of the treatments were taken at Zero time, 12 h, 24 h, 48 h and 72 h.

The optimal treatment was the addition of phage cocktail with spices mixture. The numbers of *S. aureus* cells were reduced after 12 h from the addition by 0.5 log CFU/g, after 24 h by 1.45 log CFU/g, after 48 h by 2.59 log CFU/g and after 72 h by 3.29 log CFU/g as well as *E. coli* cells were reduced after 12 h from the addition by 0.70 log CFU/g, after 24 h by 2.28 log CFU/g, after 48 h by 3.40 log CFU/g and by 4.15 log CFU/g after 72 h and *K. pneumoniae* cells were reduced after 12 h

from the addition by 1.51 log CFU/g, after 24 h by 1.90 log CFU/g, after 48 h 1.63 log CFU/g and by 3.34 log CFU/g after 72 h.

There are five treatments at 4^{o}C:

1- Ten g sterilized fresh meat was left without any treatment.
2- Ten g sterilized fresh meat was inoculated with 1 mL (10^6 CFU/mL) *S. aureus*, *K. pneumoniae* and *E. coli* bacterial cultures.
3- Ten g sterilized fresh meat was inoculated with 1 mL (10^6 CFU/mL) bacterial cultures followed by addition 1 mL of phage cocktail(1:1:1 of 10^{10} PFU/mL of each *S. aureus*, *K. pneumoniae* and *E. coli* phages).
4- Ten g sterilized fresh meat was inoculated with 1 mL (10^6 CFU/mL) bacterial cultures followed by addition of 1 mL of phage cocktail (1:1:1 of 10^{10} PFU/mL of each *S. aureus, K. pneumoniae* and *E. coli* phages, then adding 1 g spices.
5- Ten g sterilized fresh meat was treated with 1 mL of phage cocktail followed by inoculation 1 mL (10^6 CFU/mL) bacterial cultures in addition to 1 g spices.

- Aliquots of the treatments were taken at Zero time, 5 days, 10 days and 15 days.

The optimal treatment when phage cocktail was added before bacterial isolates inoculation and incubated for 30 min at RT in the presence of spices mixture. The numbers of *S. aureus* bacterial count was reduced by 2.71 log CFU/g after 5 days, by 4.14 log CFU/g after 10 days and after 15 days by 6.75 log CFU/g as well as *E. coli* bacterial count was reduced by 1.29 log CFU/g after5 days, by 6.08 log CFU/g after10 days and after 15 days by 5.16 log CFU/g and *K. pneumoniae* bacterial count was reduced by 1.55 log CFU/g after5 days, by 4.8 log CFU/g after10 days and after 15 days by 4.7 log CFU/g.